MEIOFAUNAL COMMUNITIES AND HUMAN IMPACTS AT CASEY STATION, ANTARCTICA

by

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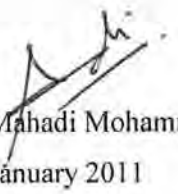

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ABSTRACT

Marine benthic communities, including meiofauna, have commonly been used as a focus of monitoring programs and of research into the effects of human activities in the marine environment. In Antarctica, benthic communities have been shown to be good indicators of human impacts, however, there is very limited information on Antarctic meiofaunal communities and how they may respond to anthropogenic disturbances. The main types of contamination present in marine sediments around Antarctic stations are metals and hydrocarbons.

A survey of sediment meiofaunal communities was done at Casey Station, Antarctica, with sampling at a range of spatial scales, from 10 meters to kilometers, to determine the spatial patterns of community composition and abundance. This included a comparison of control and disturbed areas (adjacent to old waste disposal sites). An MDS of all 47 samples supported by one way ANOSIM (Global $R= 0.955$, $P< 0.001$) showed the variation within locations was less than the variation between locations (kms) and significantly different between control and polluted locations. From the total meiofauna, a higher percentage of nematodes, by comparison to harpacticoid copepods in both controlled (nematode, 94.8%: harpacticoid copepods, 5.2%) and disturbed locations (nematode, 95.4%: harpacticoid copepods, 4.6%).

Multivariate biological (meiofaunal communities) and environmental datasets were examined to determine whether there were any correlations between patterns of

community composition and environmental variables. The analysis suggested that the most influential variables on the community pattern were metals of anthropogenic origin such as tin, lead, iron, copper, and zinc but also metals that probably relate to local differences in mineralogy such as silver, barium, uranium and arsenic. Grain size parameters were found to have a much lower capacity to explain differences in meiofaunal communities, although there did appear to be some influence.

An experiment was setup in which four different hydrocarbons (SAB diesel fuel, and clean, used and biodegradable lubricant oils) were added to defaunated marine sediments and deployed in trays in a sheltered marine bay. The communities colonizing the sediments were monitored for up to five years. The effects of hydrocarbon contamination on meiofaunal communities were different for each type of hydrocarbon. The Control and Biodegradable treatments had the most similar meiofaunal communities at all sampling times. Effects of hydrocarbon treatments were still evident after five years. Results also suggest that changes in nematode composition are ideal for long term pollution monitoring. By comparison, copepods appeared to be less sensitive to hydrocarbon pollution. Long-term monitoring is essential to understand the true extent to which lubricants impact the community structure.

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1.0 GENERAL INTRODUCTION

The Antarctic continent is considered the most pristine place in the world due to its remoteness and distance from inhabited regions of earth. This continent is far from major human activities and sources of pollution. Nevertheless, this region is susceptible to pollution caused by past and present human activities. There are more than 60 research stations in Antarctica which are managed by at least 30 countries, which are all signatory to the Antarctic Treaty. These research stations either operate during summer or all year round. Having research stations all around the continent may increase the risks of human pollution to the environment. Thus, countries adhering to the Antarctic Treaty have accepted the Protocol on Environmental Protection, often referred as the Madrid Protocol. These measures include establishing monitoring programs for waste disposal, contamination by oil or other hazardous toxic substances, construction and operation of stations, conduct of science programs, recreational activities and activities affecting the purposes of designated protected areas.

There are three permanent Antarctic stations which are maintained by the Australian Antarctic Division. Casey Station ($66^{o}17'S$, $110^{o}32'E$, East Antarctica) is the closest of the permanent Australian Antarctic stations, situated 3430 km south of Hobart. This station can accommodate up to 70 personnel over summer month and 20 over winter. To assess the impact of this station, the Australian Antarctic Division (AAD) has established an extensive environmental monitoring program in regards to human activities and its impact on the environment.

1.1 Human impacts in Antarctica

The exploration and research of Antarctica have led to some significant although often localized impact on the Antarctic environment (Aislabie et al., 2004). Several impacts arise from human activities in Antarctica including oil spills, construction, deposition of waste, introduction of alien species and disturbance to wildlife. Many of these impacts have occurred on the small areas of ice-free ground, where the majority of Antarctic scientific stations are located. Apart from environmental impacts by the station, tourism may also contribute to human impacts in Antarctica. Ice free areas are the focus of human activity and continue to attract scientists and increasing numbers of tourists. This increase in the number of voyages to the continent is likely to increase the risk of pollution by vessels.

While Antarctica has so far been relatively free from major petroleum spills, large spills are likely to happen at sea in the future and these may spread onto nearby shorelines, and involve fuel oils for ship and station use. The last catastrophe was when *Bahia Pariaso*, which ran ashore near Palmer Station on the Antarctic Peninsula, spilling about 600,000 litres of fuel (Lee and Page, 1997). Direct spillage can also occur on land, at stations during delivery, storage and use of petroleum fuels. In 1990, a spill of 91000 litres of SAB diesel fuel occurred from a fuel storage facility at Casey Station (Deprez et al., 1999). Based on observation of fuel spills at Casey Station, Antarctica, every 1 kg of fuel spilled creates between 100 to 1000 times that amounts of contaminated soil by mass (Filler et al., 2008). Although, smaller volumes are involved, petroleum spills have often occurred in environmentally sensitive areas

(Kerry, 1993). The main sources of hydrocarbon contamination in Antarctic coastal marine environments are shipping operations near the scientific stations where fuel is transported and refuelled, and also human activities such as vehicle use, fuel storage, transfers and waste disposal.

Few studies on human impacts have been conducted on the marine environment of Eastern Antarctica and most have been conducted in shallow waters (Stark et al., 2003c, Stark, 2000, Thompson et al., 2003, Stark et al., 2004). Several studies have been conducted in areas near Casey Station, investigating the contamination and human impacts due to the station operations. Previous studies in the area have focussed on how benthic microbial (Powell et al., 2005), diatom (Cunningham et al., 2003) and infaunal communities (Thompson et al., 2007) responded to metal and petroleum hydrocarbon pollution. Stark et al. (2003c) assessed the recruitment and development of soft-sediment assemblages from hydrocarbon-contaminated marine sediments, and found that there was a significantly reduced crustacean abundance in these sediments (i.e., gammarid, ostracods, tanaids and copepods) in comparison to a reference site. Stark et al (2003a) provided fundamental baseline information of disturbed and undisturbed sites at Casey Station. This study was to determine the appropriate scales and levels of spatial replication, the most suitable level of taxonomic resolution and influence of data transformation in shallow marine (max 20 metres) infaunal assemblages at Casey. Variations in populations were found at smallest scale, between replicates core and also at the level of location. Meanwhile, the patterns of assemblage structure were found similar only at fine and medium levels of taxonomic resolution. Stark et al (2003a) found that identification of differences between control and impacted areas can be done at coarse levels (phyla)

of taxonomic resolution. This was supported the findings of other studies that suggested analyses at higher taxonomic levels are useful to pollution monitoring programs (Warwick, 1988, James et al., 1995). Correlations between spatial distribution of soft sediment assemblages and environmental variables (heavy metals, sediment grain size and total organic carbon) at Casey Station were done by Stark et al. (2003b). Impacted locations (Wilkes, Brown Bay, Shannon Bay and Wharf) were characterised by fewer taxa, lower diversity and lower species richness. Sparkes Bay, one of the control locations in the study also had concentrations of cadmium and nickel, low abundances, diversity and richness similar to the impacted locations. Combination of toxicity and sediment anoxia caused by high TOC levels and fine sediment contributed by large amounts of perennial kelp (*Himantothalus sp.*) were found influencing assemblages at Sparkes Bay. In addition, study by Cunningham et al. (2003)using benthic diatoms as pollution indicators have also been conducted at Casey. The research focused on the effects of hydrocarbons on diatoms, where, results showed that diatom community composition within Brown Bay at Casey was significantly related to metal concentration (Cunningham et al., 2003). The responses to metal contamination of the diatoms were species specific, with different species showing different tolerance levels.

1.2 Marine Meiofauna

Meiofauna are one of the richest and most diverse aquatic communities in marine benthic ecosystems. They occur in both freshwater and marine habitats, from shorelines to the deep sea, and from tropics to polar regions. The marine meiofauna contains numerous undescribed species and higher taxa.

The term meiofauna is derived from the Greek *meio* meaning “smaller” (Higgins and Thiel, 1988). In this context, it refers to the fauna that are smaller than what has been defined as the lower size limit for macrofauna, i.e. less than 500 μm. Meiofauna are thus smaller than macrofauna but larger than microfauna (maximum size 32 μm). The size range separates a discrete group of organisms whose morphology, physiology and life history characteristics have evolved to exploit the interstitial matrix of marine soft sediments (Kennedy and Jacoby, 1999). Meiofauna are found in all types of sediments from softest muds to the coarsest gravels. Meiofauna can also occupy space several centimetres above sediment habitats, including rooted vegetation, moss, macroalgae, sea ice and various animal structures, e.g. coral crevices, worm tubes, echinoderm spines (Higgins and Thiel, 1988). These communities play an important role in sediment bioturbation and recycling of organic matter. They are also closely linked to communities of primary producers as they are consumers of benthic microalgae (Hack et al., 2007).

1.3 Components of the meiofaunal community

The meiofaunal community is comprised of 20 phyla (Table 1.1). Among these phyla, Nematoda, Copepoda, Rotifera, Gastrotricha, Kinorincha and Tardigrada are common in meiofaunal community. Although meiofauna consist of numerous groups, in this study, two main dominant groups were examined. These are the Nematoda and Copepoda. Within this community, Nematoda has shown a distinct dominance in comparison to other groups (de Skowronski and Corbisier, 2002, De Leonardis et al., 2008, Urban-Malinga et al., 2005, Vanreusel et al., 2000). For example, more than 60% of each community was made up of nematodes in Martel Inlet, King George Island, Antarctica (de Skowronski and Corbisier, 2002).

1.3.1 Nematodes

Nematodes are abundant in marine sediments, dominating the coastal, and sub-littoral and estuarine marine meiofauna, inhabiting even the deepest ocean trenches. They move easily through mud and sand, but are poorly adapted to swimming so that they do not occur in the plankton except as parasites or commensals of other animals. Nematodes regularly dominate the meiofauna in the top 5 cm of sediment biotopes, comprising more than 50% of the total meiofauna abundance.

Table 1.1: Currently, 20 phyla (bold) considered to be meiofaunal from the 34 recognized phyla of the Kingdom Animalia. *Source : International Association of Meiobenthologists.*

Phyla of the Kingdom Animalia				
Phyla	**Free-living**			**Symbiotic**
	Marine	**Freshwater**	**Terrestrial**	
Porifera	Yes	Yes	No	No
Placozoa	Endemic	No	No	No
Cnidaria	Yes	Yes	No	Yes
Ctenophora	Endemic	No	No	No
Platyhelminthes	Yes	Yes	Yes	Yes
Orthonectida	No	No	No	Endemic (Marine)
Rhombozoa	No	No	No	Endemic (Marine)
Cycliophora	No	No	No	Endemic (Marine)
Acanthocephala	No	No	No	Endemic
Nemertea	Yes	Yes	Yes	Yes
Nematomorpha	No	No	No	Endemic
Gnathostomulida	Endemic	No	No	No
Kinorhyncha	Endemic	No	No	No
Loricifera	Endemic	No	No	No
Nematoda	Yes	Yes	Yes	Yes
Rotifera	Yes	Yes	Yes	Yes
Gastrotricha	Yes	Yes	No	No
Entoprocta	Yes	Yes	No	Yes
Priapulida	Endemic	No	No	No
Pogonophora	Endemic	No	No	No
Echiura	Endemic	No	No	No
Sipuncula	Yes	No	No	No
Annelida	Yes	Yes	Yes	Yes
Arthropoda (Copepoda, Halacaroidea, Ostracoda, Mystacocarida, Tantulocarida)	Yes	Yes	Yes	Yes
Tardigrada	Yes	Yes	Yes	No
Onychophora	No	No	Endemic	No
Mollusca	Yes	Yes	Yes	Yes
Phoronida	Endemic	No	No	No
Bryozoa	Yes	Yes	No	No
Brachiopoda	Endemic	No	No	No
Echinodermata	Endemic	No	No	No
Chaetognatha	Endemic	No	No	No
Hemichordata	Endemic	No	No	No
Chordata	Yes	Yes	Yes	Yes

Due to the lack of knowledge regarding many nematodes, their systematic are contentious. Traditionally, the Class Nematoda is divided into two classes, the Adenophorea and the Secernentea, and initial DNA sequence studies suggested the existence of five clades: Dorylaimia, Enoplia, Spirurina, Tylenchina and Rhabditina (De Ley and Blaxter, 2004).

The Nematode body is essentially a tube within a tube. The external wall consisting externally of a cuticle layer and internally of a longitudinal muscle layer. The buccal cavity and sensory organs such as amphids and setae are important for taxonomic identification. Other important parts which are taxonomically useful are the nervous, excretory and reproductive systems (Warwick and Clarke, 1998).

1.3.2 Copepods

In terms of meiofauna abundance, the Copepoda are second to the Nematoda in sediments and probably exceed them in phytal habitats (i.e: seagrass or algal beds). There is considerable species diversity and copepods inhabit all available benthic habitats in the sea, freshwater and inland saline waters, e.g. among mosses and bromeliads, particularly in warm climatic regions (Wells, 1978). In sediments, they tend to be found on or just beneath the surface of muds but also extend deep within sands and gravels to the level of the permanent water table. In the sea they are associated with sessile epibenthic macrofauna and are especially abundant and diverse

on macrophytes, where they form a large part of the phytal meiobenthos (Wells, 1978).

Each part of the copepod body is important for their taxonomic identification. The body consists of three tagmata: the cephalosome, the metasome and the urosome. The cephalosome consists of the head and first thoracic segment. The tergites and pleurites of these segments are fused together to give a continuous head shield. The cephalosome bears the head appendages antennule (first antenna), antenna (second antenna), mandible, maxillule (first maxilla), maxilla (second maxilla) and the maxilliped of the first thoracic segment. In most harpacticoids, the second thoracic segment has become fused with the cephalosome to form a cephalothorax. The metasome primitively consists of the thorax for the first segment, and bears the first five pairs of “swimming legs”, or pereiopods (Wells, 1978).

The life cycle of copepod can determine their ecology. Their planktonic stages includes six nauplius larval and five subadult copepodid stages during which there is a progressive addition of prosome and ursome segments and development of their appendages. During these stages, copepods are only found floating and swimming in the water column.

The sexes can be distinguished by the fourth copepodid stage (Huys et al., 1996). All species are sexually dimorphic but the only universal morphological difference is the structure of the first two abdominal segments. In the female, these are fused into a

genital somite and the sixth pereiopod is reduced to one or two setae flanking the single, median genital aperture. In the male the two segments remain separate and the sixth pereiopod is far more elaborate than the female. Females are usually larger than males. Other, almost universal sexual differences occur in the male antennule, which usually is prehensile to a greater or lesser degree, and the fifth pereiopod, which usually is smaller and less elaborate than the female. Sexual dimorphism may also be apparent in pereiopods 1 to 4 but there is great variability in the form of such differences between, and even within, families. There are currently ten groups of copepods. Three main groups, Calanoida, Cyclopoida, and Harpactacoida are free-living and most likely to be encountered. The Calanoida and Cyclopoida are primarily planktonic. The Harpactacoida are primarily benthic, as evidenced by their vermiform (worm-shaped) bodies. At present the Order Harpacticoid copepods contains about 2757 species distributed among 346 genera in 33 families. Harpacticoid copepods range in size from 0.2 mm to 2.5 mm (Giere, 1993) and thus can all be classed as meiofauna.

1.4 Patterns of meiofauna distribution

Some macrofauna are a part of the meiofauna only during their juvenile stages, but many taxa contain species that are meiofauna throughout their life cycles. This permanent meiofauna includes the Mystacocarida and many representatives of Rotifera, Nematoda, Polychaeta, Copepoda, Ostracoda and Turbellaria. Many studies have shown that the distribution patterns of meiofauna are affected by factors such as the sediment type, depth and food availability (de Skowronski and Corbisier, 2002,

Veit-Köhler et al., 2008, Gutzmann et al., 2004). A high abundance of nematodes was found to be positively correlated with the presence of a high abundance of cyanobacteria (Doulgeraki et al., 2006). A higher percentage of meiofauna biomass is recorded from brackish water,, intertidal beaches and from the deep sea, where meiofauna and macrofauna biomass are of the same magnitude (Gerlach, 1971). There are approximately 10^6 m^{-2} meiofaunal organisms in almost every natural (uncontaminated) estuarine sediment worldwide and a dry weight standing crop biomass of 0.75 – 2.0 gm^{-2} (Coull and Bell, 1979).

Certain taxa are restricted to particular sediment types. Sediments where the median particle diameter is below 125 μm tend to be dominated by burrowing meiofauna (Coull, 1988). The interstitial groups, for example the Gastrotrichia and Tardigrada, are typically excluded from muddy substrates where the interstitial lacunae (cavity) are closed. The sand fauna tends to be slender as it must manoeuvre through the narrow interstitial openings, whereas the mud fauna is not restricted to a particular morphology but is generally larger (Coull and Bell, 1979). In general, sediment grain size is a primary factor affecting the abundance and species composition of meiofaunal organisms. For example the genus *Astomonema*, *Terschellingia*, *Theristus*, *Sabatieria* and *Dorylaimopsis* were found to be highly dominant in muddy sediment and to have a low abundance in sands (De Leonardis et al., 2008).

Benthic nematodes are among the primary consumers of bacterial. Moens et al (1999) concluded that selective recruitment to food spots may be a major factor driving the heterogenous field distribution of bacterivorous nematodes. Gray and Johson (1970)

documented that bacterial films on sand grains differently attract meiofaunal organisms. Moens et al (1999) demonstrates a highly species specific marine nematode (*Monhystera* sp.) preference to Gram-negative bacterial. However, Warwick (1981) found that the occurrence of a species in a specific biotope is not only determined by its feeding behaviour, but also factors such as reproductive capacity, tolerance to environmental conditions, competition and predation, which all play roles in the survival strategy of nematode species (Bouwman, 1984). This is because, same taxa have the ability to switch their diet when a specific food items is limited (Moens and Vincx, 2009) or when the quality of organic matter available to the deposit and epistratum-feeders changes with season. In the study by Da Rocha et al. (2006), they found that presence of nematode not only influenced by food availability but also the complexity and type of habitat. For example, they suggested that *Halalaimus sp.* showed the specificity based on habitat type as this species was found almost restricted (94%) to in habitat dominated by *Hypnea musciformis* and *Padina gymnospora*.

In almost all meiofauna studies, the majority of the fauna has been found in the upper 2 cm of sediment. Vertical zonation is controlled by the depth of the Redox Potential Discontinuity (RPD) level, i.e. the boundary between aerobic and anaerobic sediments. The primary factor responsible for vertical gradients in the RPD is oxygen (McLachlan (1978), which determines the redox potential as well as the oxidation state of sulphur and various nutrients. When redox potentials are low, meiofauna densities greatly decrease.

Harpacticoid copepods are typically the most sensitive meiofauna taxon to decreased oxygen, and are usually restricted to oxic sediments (Wieser et al., 1974). Some meiofauna appear to be capable of tolerating low or no oxygen conditions and thus penetrate sediments below the RPD (Reise and Ax, 1979). There is however, a debate over how these animals adjust to reduced O_2 levels (Schiemer et al., 1990) and whether the occupied habitats below the RPD are truly anoxic (Boaden, 1980). In mud and sediments heavy in detritus, meiofauna are often restricted to the upper few mm or cm of oxidized sediments (Coull and Bell, 1979). Most of the research on vertical patterns of meiofauna vertical distribution has been conducted in sandy substrata (Gheskiere et al., 2004, Gheskiere et al., 2005, Rodri´guez et al., 2003, Nicholas and Hodda, 1999) and deep sea (Vanreusel et al., 1995, Steyaert et al., 2003). In sands the meiofauna can be distributed to the depth of the RPD, which on high energy beaches can be 50 cm or more deep (Higgins and Thiel, 1988). Oxygen content is the ultimate factor controlling vertical distribution of meiofauna in beaches (McLachlan, 1978).

Meiofauna are also known to be sensitive to low pore water content (Jansson, 1968). As sand dries at low tide, the fauna face desiccation stress despite the oxygen content. McLachlan et al. (1977) found that meiofauna migrated downwards on an ebbing tide and upwards on a flooding tide. Vertical migration is typically less in winter than in summer and this appears to be related to lower winter temperatures and therefore less desiccation at low tide than in the summer. Furthermore, vertical migration is reduced at night, probably in response to cooler night temperatures at low tide and again, less desiccation. Thus, the migrations are not entirely dependent on the tides since desiccation varies seasonally and diurnally.

It is well known that environmental disturbance affect the distribution of organisms in a given ecosystem. The abundance and distribution of meiofaunal communities are also influence by natural disturbance such as bioturbation (Sellanes and Neira, 2006), storms (Peck et al., 1999), tidal effects (Gheskiere et al., 2006) and iceberg scouring (Gutt and Piepenburg, 2003, Gutt et al., 1996, Lee et al., 2001, Gerdes et al., 2003, Peck et al., 1999). Among the natural disturbance, influence by icebergs scouring is the important in the polar region. Whereby, icebergs affect soft substrata in three main ways. Firstly, they plough the seabed that forces surface layers away from the point of contact; second, they crush it, whereby icebergs rock backwards and forwards crushing underlying organisms and seabed; and finally, water flowing around icebergs either caused by movements of the berg, natural oceanic currents, or salinity induced water actions can resuspend and transport sediment (Reimnitz et al., 1977). These changes will subsequently alter the habitats, sediment properties and food availability. In any disturbance there is an immediate change in the abundance and diversity of meiofauna but there is subsequent recovery. When an area occupied by a set of species is disturbed, re-colonization and succession will occur with a new set of species (Mani et al., 2008). For example, Raes et al (2010) noted that nematodes appear to be strongly influenced by the sudden removal of ice-cover in the Antarctic Larsen ice shelf area. Raes et al (2010) indicate that pre-collapse, sub-ice communities were impoverished and characterized by low densities, low diversity and high dominance of a few taxa. However, an increase in food supply after ice-cover removal provoked a fast, local response of the nematode assemblages to recolonization. The collapse of the ice shelves showed a positive effect on the shelf nematode fauna in the area, both in terms of abundance and diversity. In a study by Kotwicki et al (2004), they found that glacial runoff (freshwater input) and enhanced

sedimentation rates at Kongsfjorden, Spitsbergen reduces the number of individuals in the meiofaunal community.

Apart from recolonization of species as a response to disturbance, these communities are also able to adapt to the changing environment. For example, Gheskiere et al (2005) suggested that nematodes in dynamic environments usually exhibit morphological adaptations (such as body ornamentations which provide an anchorage) to high turbulence and shifting sediments. Seasonal changes may also alter the abundance and biomass of the community. In a study by Rudnick et al (1985), they observed that highest abundances and biomass occurs in May and June, while lowest values were in late summer and autumn. In springtime increases of meiofauna were observed. They concluded that microalgae detritus accumulated in the sediment during winter and early spring, and the meiofaunal responded to this store of food when temperatures rose rapidly in the late spring.

1.5 Past research on meiofauna in Antarctica

Early studies of marine nematodes from Antarctic waters, were based on collections made by various national expeditions between 1882 and 1931 (Platt and Warwick, 1983, De Broyer et al., 2007). In the Atlantic sector of the Antarctic Ocean, material from shallow sublittoral areas of South Georgia and the west coast of the Antarctic Peninsula were obtained from diving and grab-sampling in deeper waters off South Georgia and in the Weddell Sea. It is suggested that detailed studies of nematode

communities could provide a valuable method of addressing some of the classical aspects of Antarctic biology.

Scientific publications on Antarctic nematodes are scarce. The first nematode to be described from Antarctic waters was *Deontostoma antarcticum,* collected at South Georgia during the German International Polar-Year Expedition (1882-1883). Descriptions of meiofauna collected by various national expeditions include the following. Initially, 13 nematodes were collected and described by Larsen's Ross Sea Expedition (1929-1930) from Macquarie Island and in the Ross Sea (Allgen, 1930). Other report of nematodes are from the Antarctic Peninsula (Inglis, 1958). Linstow (1907) also provided the first description of a nematode from the East Antarctic *(Leptosomatum australe* from the Ross Sea). Later, Cobb (1914) described 25 new species collected in the Ross Sea by Shackleton's Expedition (1907-1909). Epsilonematidae and Desmoscolecida, collected by the German Antarctic Expedition, *The Gauss Expedition* (1901-1903), were described by Steiner (1931b, 1931a) and Timm (1970) respectively. Mawson's two Antarctic expeditions (1911-1914, 1929-1931) provided material which resulted in the valuable contributions of Cobb (1930) and Mawson (1958a, 1958b). Further descriptions of Enoplida from Kerguelen Island were made by Schuurmans-Stekhoven and Mawson (1955) and Platonova (1958). Studies involving nematodes also have been reported from the Ross Sea (Hope, 1974, Timm and Viglierohio, 1970), Kerguelen Island (Arnaud, 1974, de Bovee and Soyer, 1975) and Weddell Sea (Vanhove et al., 1999, Herman and Dahms, 1992, Vermeeren et al., 2004, De Mesel et al., 2006, Fonseca et al., 2006)

A detailed history of copepods recorded from Antarctica by several expeditions was written by Golemansky and Chipev, (1999) in their book entitled "*Bulgarian Antarctic Research. Life Sciences*". In this book, it was stated that the first records of harpacticoid copepods from the Antarctic were given by Giesbrecht (1902), who described the copepods collected during Belgica Expedition (1897-1899). It were then followed by Brady (1910), who worked on the material of the German Southpolar Expedition (1901-1903) and discovered *Harpacticus simplex, Mesochra nana*, *Laophonte gracilipes* and *Amphiascus minutes* during the expedition. Later, Lang (1936) described a new species, *Amphiascus gracilis,* from the same material. Harpacticoid copepods have also been collected by the British Antarctic Expedition (1902-1904) and the French Antarctic Expedition (1903-1905). The materials of the French Expedition were identified by Quidor (1920) who reported three harpacticoid species of the genus *Porcellidium*. Golemansky and Chipev, (1999) discussed the species composition of harpacticoids collected by the Australian Antarctic Expedition (1911-1914) that included seven harpacticoid species from the region of Kerguelen and St. Paul Island and were published by Monard & Dollfus (1932), Lang (1934) and Brady (1918).

There have been many studies reporting the distribution of harpacticoid copepods in Antarctica, such as; the Weddell Sea (Schnack-Schiel et al., 2001, Günther et al., 1999, Dahms et al., 1990, Herman and Dahms, 1992, Vanhove et al., 1995, Dahms, 1989), Ross Sea (Bradford and Wells, 1983, Gambi et al., 2004), Amery Ice Shelf (Swadling et al., 2000), Vestfold Hills (Swadling, 2001), Langhovde (Kudoh et al, 2008), Syowa Station (Hoshiai et al., 1996), King George Island (Veit-Kohler and Fuentes, 2007), Kerguelen (Huys and Conroy-Dalton, 2006).

Studies carried out in the Weddell Sea confirmed the presence of a relationship between meiofauna distribution and food indicators (Lee et al., 2001) but also reported high meiofaunal densities compared to similar deep environments at temperate latitudes (Herman and Dahms, 1992). Studies have shown that the meiofauna community shows a pattern of decreasing densities with increasing water depth (Gutzmann et al., 2004, De Leonardis et al., 2008, Vanhove et al., 1995), which is related to a reduction in organic matter and food availability. Although meiofauna in polar regions show a large spatial variability (Vanhove et al., 2000) the parameters which control meiofauna distribution and community structure are still unclear.

A study by Fabiano and Danovaro (1999) on meiofauna distribution in the Ross Sea described different trophic and sediment characteristics and indicated that at macro-scales (kilometres) meiofaunal communities are dependent on particulate organic matter fluxes. At micro-scales (centimetres) a very low variation in meiofauna density contrasted with large meso-scale (metres) variability, which was related to the concentration of the main food indicators such as phytopigments, proteins, carbohydrates and lipids.

1.6 Meiofauna as an indicator species

Marine benthic organisms and communities have commonly been used as a focus of monitoring programs(Tin et al., 2008) and in research into the effects of human activities in the marine environment (Platt and Warwick, 1983). Environmental stress,

such as pollution, is considered to decrease marine benthic organism species diversity i.e. total number of species or taxonomic groups (Warwick and Clarke, 1995, Clarke and Warwick, 1994). They are used in monitoring programs due to their relative lack of mobility and their trophic position (Kennedy and Jacoby, 1999, Gomez Gesteira et al., 2003). Firstly, these sedentary organisms are less mobile and this will reduce their chance of avoiding potentially harmful conditions (Sutherland et al., 2007) and secondly, nearshore benthic organisms are often closely coupled with pelagic food webs, constituting a link for the transport of contaminants to higher trophic levels (Coull, 1990).

Changes in benthic faunal communities have been used as indicators of contamination and pollution in marine ecosystems (Simboura and Zenetos, 2002, Kennedy and Jacoby, 1999, Bustos-Baez and Frid, 2003, Kennish, 1998). Meiofaunal communities have been considered better indicators of pollution and human impacts in the marine benthic environments than macrofauna communities as they have a shorter life cycle and are more sensitive to environmental changes. As a direct benthic developer, meiofauna stay in the sediment throughout their life cycle. Furthermore, as they are smaller and have limited mobility they are ideal organisms as pollution indicators. As meiofauna are also in constant contact with water in the sand they are thought to be more reliable indicators of pollution than bivalves, which siphon water from the overlying water column. Meiofauna comprise a basal component of the food web and disturbing them could have unforeseeable trophic consequences. Altered species composition could significantly influence interactions between nematodes and interactions among major benthic taxa (Mahmoudi et al., 2005, Austen et al., 1994, Austen and McEvoy, 1997). In addition, many juvenile fish and crustaceans prey on

meiofauna, particularly nematodes and copepods (Coull, 1990). Responses of free living nematodes to diesel contamination (elimination of some species, increase or decrease of some others) could lead to food limitation for juvenile fish and crustaceans which could ultimately alter entire communities and ecosystems (Warwick and Clarke, 1998).

Meiofaunal assemblages are ideal for experimentation as they are small, abundant, have a short life cycle, are easily maintained, and are sediment bound throughout their life history (Higgins and Thiel, 1988). The meiofauna are the most abundant metazoan component of marine organisms. Total meiofauna densities may exceed 1.29×10^7 individuals per square meter of sediment surface (Warwick et al., 1979) while densities of individual species may reach 5.7×10^6 m^2 (Hicks, 1988). Their close association with sediment means that changes in interstitial chemistry quickly feeds through to changes in meiofauna abundances and diversity.

Meiofaunal assemblages and, in particular nematodes have been increasingly utilized as indicators of organic disturbance because of their ubiquity, high densities and high taxonomic diversity (Mirto et al., 2002). Meiofauna possess a number of advantages over macrofauna in their suitability for data collection. The organisms are sufficiently small and numerous to allow small volume sediment cores to contain statistically adequate counts of total fauna. This means that many replicate samples can be collected by SCUBA divers without the need for a field-biased processing stage. However, Kennedy and Jacoby (1999) suggested that there were also some disadvantages in using meiofauna as pollution indicators. Meiofauna are very small,

thus, identifying these organisms is time consuming. Thus, due to lack of research and study, the availability of the identification keys is limited. Kennedy and Jacoby (1999) also concluded that the meiofauna have a high level of spatial and temporal variability in their distribution. The abundances of meiofauna are also effected by environmental factors such as salinity (Soetaert et al., 1995), sediment type (Veit-Köhler et al., 2008) and wave effects (Rodri´guez et al., 2003) and these factors will also affect the interpretation of the results.

Parker (1975) and Raffaelli and Mason (1981) have proposed using the ratio of nematodes to copepods (N/C ratio) as a monitor of pollution or sediment changes that eliminates the need for detailed, time consuming taxa identification. While, this idea is very attractive, it has generated considerable controversy (Warwick, 1981, Lambshead, 1984, Raffaelli and Mason, 1981, Platt et al., 1984, Coull et al., 1981) and it has not been proven to be an accurate predictor of environmental change. While, the technique is simple because no taxonomic experts need be sought, further research is needed to determine the universality of the N/C ratio as a measure of environmental perturbation. Because of rather difficult taxonomy of meiofauna; many perturbation studies prefer not to include them.

Previous studies have shown that meiofauna have similar responses to macrofauna to human disturbances of aquatic environments (Coull and Chandler, 1992, Peterson et al., 1996, Schratzberger and Jennings, 2002) and are very sensitive to different sources of organic contamination (Mirto et al., 2002). Peterson et al. (1996) argued that macrofaunal and meiofaunal communities display repeatable patterns of response

to environmental stressors, which are generally detectable at high taxonomic levels (even phyla). Hence, echinoderms and crustaceans, especially amphipods and some harpacticoid copepods, are highly sensitive to toxic chemicals in their environment and these groups typically show large declines in abundance due to sediment toxicity. By comparison, nematodes, polychaetes, and oligochaetes are not especially sensitive to toxins. These groups tend to include species with opportunistic life histories and less susceptible feeding types (especially non-selective deposit feeders) that render them capable of utilizing organic materials associated with organic enrichment. These taxa typically show substantial increases with organic pollution where oxygen depletion is not a factor (Peterson et al., 1996). While, nematodes are highly tolerant to stressed environmental conditions, copepods are sensitive to biodeposition (Mirto et al., 2002). This suggests that organic enrichment of sediments will alter the abundance and community structure of meiofaunal assemblages.

1.7 Research Objectives

This study had three main aims: A) To investigate meiofaunal communities in the Casey region and determine their composition, distribution and abundance and patterns of spatial variation; B) to determine whether there were any correlations between community patterns and environmental variation, including potentially impacted areas; C) to assess the effects of sediment hydrocarbon contamination on Antarctic meiofaunal communities. In order to achieve this, the study was divided into three parts (Figure 1.1). All free-living nematodes and harpacticoid copepods were identified.

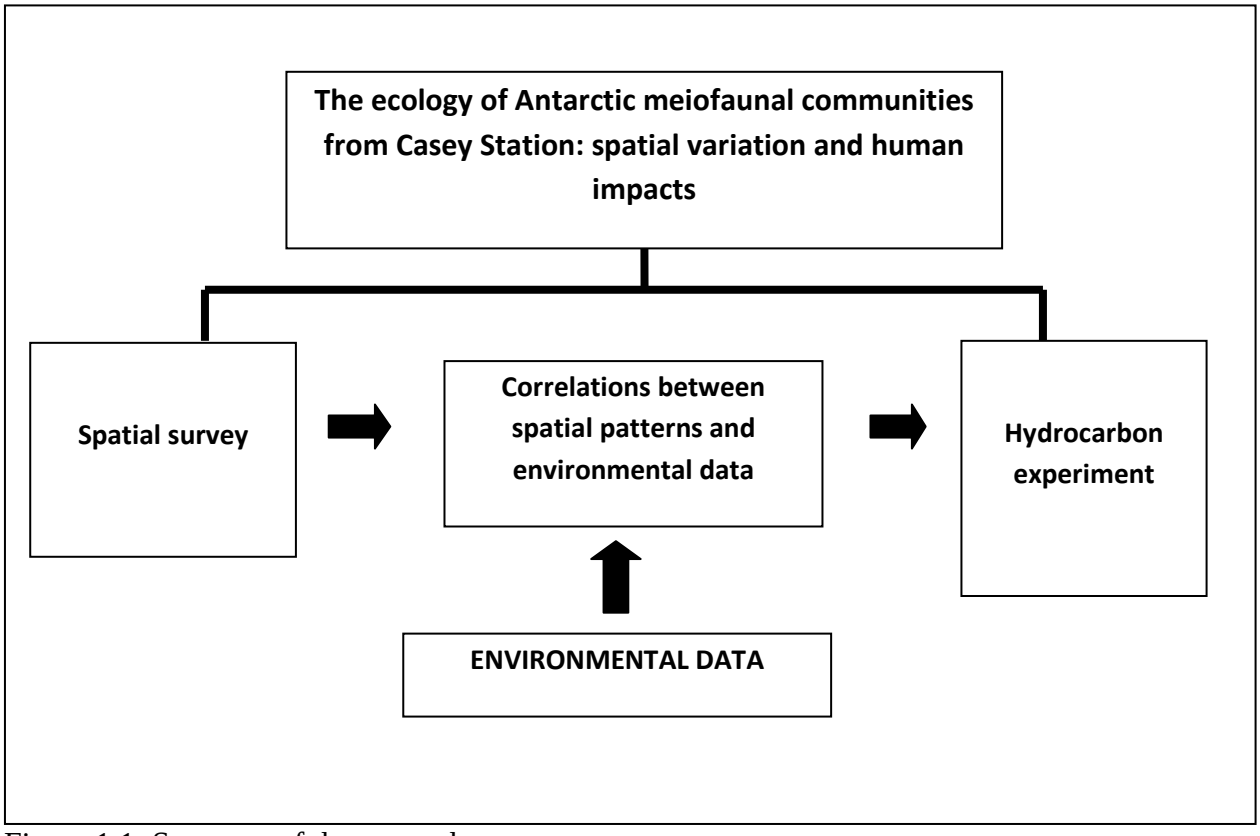

Figure 1.1: Structure of the research.

A. Spatial Survey

A survey of meiofaunal communities was undertaken to determine the spatial variation, abundance and biodiversity of meiofauna. A spatially nested sampling design was used in this survey. The design allows comparisons of variability at several scales: at ~10 m among plots within sites, at ~100 m among sites within locations and between the locations. O'Brien Bay (O'Brien Bay 1 and O'Brien Bay 5) and McGrady were considered as control locations. Surveys were also undertaken at two locations impacted by abandoned waste disposal sites. The disturbed locations are Brown Bay (Brown Bay Inner and Brown Bay Middle) and Wilkes.

The first objective of this component was to investigate and describe the spatial patterns of community composition and abundance by looking at the natural variation at different scales, from meters to kilometres. The second objective was to compare the control locations and disturbed locations (waste disposal sites).

The hypothesis that was being tested in this spatial survey study was there will be differences in meiofaunal communities between locations and within locations.

B. Correlations between spatial patterns and environmental data

The aim of this component is to examine relationships between meiofaunal communities and environmental patterns. The main environmental variables used are sediment properties such as grain size, organic carbon and metals in sediments. Multivariate biological and environmental datasets will be examined to determine whether there any correlations between patterns of community composition and environmental variables. Such relationships will suggest causal relationships between environmental variables and differences in meiofaunal community structure.

C. Hydrocarbon Experiment

The objective of this study is to experimentally investigate the responses of free-living nematode and harpacticoid copepod communities to hydrocarbon pollution. Benthic invertebrates may be continuously exposed to Polycyclic Aromatic Hydrocarbons (PAHs) in contaminated areas since PAHs are relatively insoluble in water, absorb strongly to particulate matter and accumulate in bottom sediments. Furthermore, they are a high risk and common pollutant. Thus, the aim is to demonstrate a causal link between presence of hydrocarbon pollution and environmental impacts. The experiment was setup to monitor their response change through time up to five years (260 weeks). There are five period of sampling (T1, T2, T3, T4 and T5) but in this study only T2 (54 weeks), T4 (102 weeks) and T5 (260 weeks) were monitored.

The hypotheses being tested here are:

1. There will be differences between in meiofauna communities in control and oiled treated experiments.
2. The effects on meiofaunal communities will be different among the different oil treatments used in the experiments
3. Duration of exposure to oil treatment will affect meiofaunal abundance and community composition.

2.0 SPATIAL VARIABILITY OF MEIOFAUNAL COMMUNITIES AT CASEY STATION

2.1 Introduction

The meiofauna is defined as animals passing through a 1.0 mm sieve but retained on a 32 µm mesh. However the distribution of meiofauna is strongly related to the amount of utilizable organic matter in the sediment (Fabiano and Danovaro, 1999), which is controlled by sedimentation and organic degradation rates (Doulgeraki et al., 2006). Meiofauna can stimulate microbial activity in the sediment (Tenore et al., 1977), they are food for the macrofaunal communities (Gee, 1989) and they can also be a potential bioindicator of environmental impacts.

De Leonardis et al. (2008) suggested that the abundance and distribution of meiofauna was related to water depth, whereby shallower sites having a greater abundance and diversity and deeper sites having lower densities and a lower number of taxa. Similar results were also found by Gutzmann et al. (2004), where, the abundance of nematodes and copepods decreased with increasing water depth. Doulgeraki et al. (2006) suggesting that bottom currents, sediment particle size and pH were the most important factors explaining spatial differences in meiofaunal. In addition, De Troch et al. (2001) found that at South of Mombasa Island, Tanzania the variation within meiofauna assemblage was linked to the specific habitat requirements whereby certain genus of meiofauna very only found on certain seagrass species. They concluded that meiofauna was structured by environmental conditions as selected and partly created by the seagrass species. The habitat selected by the seagrass species, in view of its

role in the succession, in terms of grain size, organic matter and pigments determines the associated meiofauna.

Coastal benthic habitats are among the most productive of marine environments (Barnard, 1998, Levinton, 1995, Borum and Sand-Jensen, 1996, Suchanek, 1994). They typically receive a rich nutrient supply, which is influenced by both terrestrial nutrient sources and coastal phytoplankton production. In Antarctica, however, terrestrial nutrient supply is extremely limited or almost entirely absent as there are no rivers and little terrestrial primary production. The oceanic waters surrounding the Antarctic continent are characterised by high nutrients levels, which often become seasonally low during the summer, but rarely to levels that could be considered limiting to the growth of phytoplankton. In Antarctic coastal areas nutrients are relatively high throughout the year and are only briefly lowered during summer (McMinn and Hodgson, 1993, McMinn et al., 1995).

The understanding of the ecology of meiofauna has increased in the last 20 years. However, most quantitative information is limited to the Atlantic (Vincx et al., 1994., Vanreusel et al., 2009, Sebastian et al., 2007, Van Gaever et al., 2004, Vanreusel et al., 1995), Pacific and Indian Oceans (Grove et al., 2006, Vincx et al., 1994, Shirayama and Kojima, 1994, Gambi et al., 2003) and to the Mediterranean Sea (Soyer, 1985, Soetaert and Heip, 1995, Danovaro et al., 1995, Pusceddu et al., 2009, De Leonardis et al., 2008, Doulgeraki et al., 2006, Vezzulli et al., 2003, Mirto et al., 2002, Moreno et al., 2009). Other studies of meiofauna include temperate coastal shores (Rudnick et al., 1985, Veit-Köhler et al., 2008), tropical seagrass bed (De Troch et al., 2001), subtropical shores (Grove et al., 2006) and mangrove ecosystems

(Armenteros et al., 2006), where they are the numerically dominant (Armenteros et al., 2006).

As far as meiofauna is concerned, very scarce information is available from polar regions. To date, only few investigations have been carried out in northern boreal waters (Bick and Arlt, 2005, Pfannkuche and Thiel, 1987), for example, the Laptev Sea (Werner and Martinez Arbizu, 1999, Vanaverbeke et al., 1997). As for Antarctica, in the Weddell Sea (Lee et al., 2001a, Sebastian et al., 2007, Herman and Dahms, 1992, Vanhove et al., 1999, Schnack-Schiel et al., 2001, Vermeeren et al., 2004, Schnack-Schiel et al., 2008) and in Ross Sea (Fabiano and Danovaro, 1999)
The structure, composition, and diversity of the Antarctic meiofauna are poorly understood, particularly in East Antarctica. There are only a few studies on meiofaunal communities in the eastern part of Antarctica (Swadling et al., 2000, Kito et al., 1996, Swadling, 2001). Kito et al. (1996) described a new species of nematode, *Eudorylaimus andrassy*, 1959 which was found among green algae near the Russian Station, the Molodezhnaya. Research in Antarctica has included distribution (Gutzmann et al., 2004, Vanhove et al., 2004, Doulgeraki et al., 2006) spatial and temporal variation (Armenteros et al., 2006), assessment of pollution (Somerfield et al., 1994), colonization (Veit-Köhler et al., 2008, Urban-Malinga et al., 2005) and relationships to environmental factors (Vanhove et al., 1995).

The distribution of meiofauna in polar regions also varies with environmental factors. Studies by de Skowronski and Corbisier (de Skowronski and Corbisier, 2002) suggest that the meiofaunal density in the shallow coastal zone in King George Island,

Antarctica is very high compared to coastal studies in other regions. Skowronski & Corbisier (2002) concluded that most meiofaunal studies in polar regions showed a pronounced dominance of nematodes, followed by copepods. Their study of King George Island (Antarctic Peninsula) found that nematodes comprised more than 60% of the meiofauna, while, Fabiano and Danovaro (1999) found that nematodes comprised 53% -80% of the meiofauna at Ross Sea, Antarctica. The explanation for this dominance is thought to be because nematodes possess an incredible ability to adapt to the most varied environmental conditions (Lee and Van de Velde, 1999, Vanreusel, 1990, Vanreusel, 1991, Vanreusel et al., 1997). Fabiano and Danovaro (1999) noted that meiofauna in two sampling location in Ross Sea, Antarctica whereby distance between sampling corers were about 300-800 metres, a large spatial variability on a scale of a few hundred meters were observed, but the parameters controlling their distribution and community structure were however unclear. In a study conducted by Gobin and Warwick (2006), they found that generally nematode abundances were lowest in polar regions (Signy Island), greater in temperate (South west England and New Zealand) and highest in tropical regions (Trinidad and Tobago). Their study also suggested that there are some similar species found in both tropical and temperate areas.

The shallow coastal benthic habitat in Casey Station consists of small areas of soft-sediment which are interspersed between patches of rocky habitat. The shallow habitats in areas that are covered by annual sea ice for much of the year are occupied by various communities dominated by sponges, ascidians, tubeworms and other invertebrates (Stark et al., 2003b). In areas with longer periods of open water and less sea ice cover, benthic communities are dominated by macroalgae. The benthic

community at Casey Station has been studied extensively, including research on the benthic diatom communities (Cunningham et al., 2005, McMinn et al., 2004) and the effect of human impacts on benthic communities (Stark et al., 2003a, Stark, 2000, Cunningham et al., 2003b, Powell et al., 2005, Stark et al., 2005).

The major aims of this study are a) to determine the spatial variability of the meiofaunal communities at three different scales: Locations (up to several kms); Sites within locations (~100 m); Plots within sites (~10 m) and b) investigate potential human impacts on meiofaunal communities, comparing three control and three contaminated locations near Casey Station. This research can be also used as a baseline for future studies and provide an overview of the meiofaunal assemblage inhabiting the shallow coastal water at Casey.

2.2 Materials and Methods

Sampling design: Sediment samples were collected by divers using small core tubes (16mm diameter) from six locations including three control locations and three polluted locations adjacent to waste disposal sites. Sampling was done using a hierarchical, three level nested design at a range of spatial scales, from 10 meters to kilometres, to determine the spatial patterns of community composition and abundance. Two disturbed locations were situated along a gradient of pollution within Brown Bay (Inner and Middle), the other represented at a single potentially impacted area (Wilkes). Within each location there were two sites (~ 100m apart) and within each site there were two plots (~10m apart). Within each plot (1m diameter), two replicate cores were taken.

2.2.1 Location Description

Casey Station

Casey Station is situated at 66^{o}17’ S, 110 ^{o} 32’E on Bailey Peninsula in the Windmill Islands, Antarctica (Figure 2.1). The shallow (< 50 m) benthic marine environment in the near shore region at Casey is very heterogeneous (Stark, 2003). In this study, the locations selected around Casey Station were Brown Bay (Middle and Inner), off Wilkes, O’Brien Bay (1 and 5) and McGrady Cove.

Brown Bay

Brown Bay is a small embayment at the southern end of Newcomb Bay with a maximum depth of approximately 20 m and with rocky sides grading to a muddy bottom (Cunningham et al., 2003b). Brown Bay is typically ice free for 1 to 2 months in a year, usually in January and February. It is situated adjacent to the abandoned Casey waste dump, which was on the foreshore of the bay, at the base of Thala Valley (Stark et al., 2003b). Samples were taken from 2 locations within Brown Bay: Brown Bay Inner was 30 m from the waste dump directly in front of the point where summer melt water from the valley enters the bay (Stark et al., 2006) and at a water depth of 7 m. Brown Bay Middle was 150 m from the waste dump and ranged from 12 to 15 m in depth.

Wilkes

The first Australian station in the area, Wilkes was built by the USA in 1957 on Clark Peninsula on the shore of Newcomb Bay but was abandoned in 1969 (Cunningham et al., 2005). The Wilkes waste dump site is on the foreshore of a small embayment on the northern side of Newcomb Bay. It has a gently sloping bottom and the sampling site was in approximately 15 m water. The marine benthic habitat consists of coarse sandy sediments and areas of exposed rock covered with microalgae in summer. Samples were collected from approximately 50 m directly in front of the waste dump.

O’Brien Bay

O’Brien is a large bay situated several kilometres south of Casey station. This site is clean and unlikely to be contaminated by human activities. Therefore, the area was selected as a reference location. The benthic habitat consists of rocky bottoms, boulders, cobbles and gravel overlain by small patches of sediment (Stark et al., 2003a). Samples were taken in approximately 15–20 m of water.

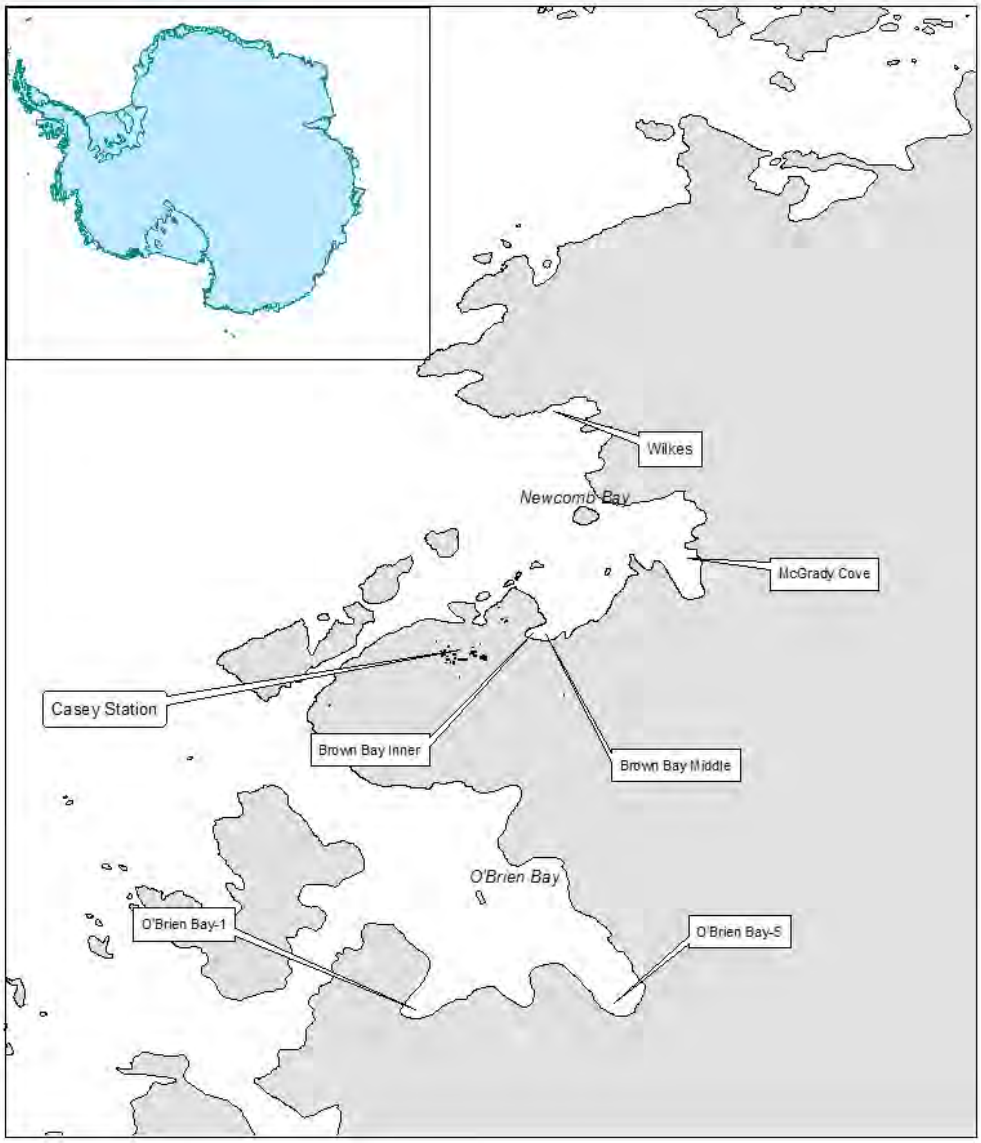

Figure 2.1: Map shows the location of spatial survey study

2.2.2 Meiofauna preparation and identification

The preserved sediment was initially sieved through a 500 µm sieve to remove the coarser fraction. Meiofauna taxa were extracted from muddy sediment through a modified gravity gradient centrifugation technique (Heip et al., 1985, Pfannkuche et al., 1988) using Ludox. Ludox HS40 and Ludox AS both have a density greater than the density of meiofauna, i.e. 1.08 for nematodes, but similar for all hard-bodied meiofauna (Witthoft-Muhlmann et al., 2005). Ludox is a silicasol (a colloidal solution of Si02) which causes no plasmolysis. Ludox HS40 is toxic and so could only be used for preserved material. Ludox AS is not toxic and could be used when living meiofauna had to be separated from sediments. For both types of Ludox, a 50% solution in distilled water is used (density of 1.15). Samples were rinsed thoroughly over a sieve of 32 µm with tap water to prevent flocculation of Ludox,.

The samples were transferred from the sieve to a large centrifuge tube. Ludox solution (60% Ludox and 40% water; density = 1.18) was added to tube until the level of mixture was balanced for centrifuging. The sample was then centrifuged at 2800 rpm for 10 min. The supernatant was decanted and collected, and the remaining sediment pellet was resuspended. This process was repeated three times. All supernatants were filtered through a 100 µm sieve, followed by a 32 µm sieve. The supernatant was finally rinsed through a 32 µm sieve with tap water to avoid the reaction between the Ludox and formalin which forms a white gel which is difficult to remove. After the extraction, 4% formaldehyde was added to the treated sample. The organisms retained on the 32 µm sieve were fixed in 4% formaldehyde. The sample was stained with 1% of Rose Bengal to facilitate counting.

All animals retained on the 32 µm sieve were counted and sorted into major taxa. The major taxa (Nematoda and Copepoda) were counted using a dissecting microscope at 25X magnification (Zeiss Stemi 2000; Zeiss Inc., Germany). Per sample, 200 nematodes (or all nematodes when density less than 200 individuals.) were picked out at random and mounted on slides in glycerine after a slow evaporation procedure (modified after Riemann, 1988). For identification to genus level Platt and Warwick (1983, 1988) and NeMys online identification (Steyaert et al., 2005) were mostly used. All copepods were picked out and mounted on slides in glycerine without evaporation for identification to family level using THAO: Taxonomische Harpacticoida Archiv Oldenburg 2005 and Bodin (1997). The identification of nematodes and copepods was done using 1000 times magnification.

2.2.3 Statistical methods

Univariate analysis of variance (ANOVA) was used to analyse meiofaunal abundances in sediments. Analysis was done using the program GMAV5 (Underwood and Chapman, 1998). A hierarchical spatially nested structure was used within the asymmetrical analysis and the separate ANOVA from which it was constructed. The sums of squares for the asymmetrical analyses were taken from three factor ANOVA (location, site nested within location, and plot nested within site). Cochran's test was used to test for homogeneity of variances in ANOVAs. If variances were heterogeneous, data were transformed (Snedecor and Cochran, 1980, Underwood, 1981). Where significant heterogeneity of variances could not be removed by transformation, a lower significance level of $P = 0.01$ was used. When a significant

effects were encountered, post-hoc multiple comparisons among means using the Student–Newman–Keuls (SNK) test (at a=0.05) were carried out. Species Richness (S), Shannon DiversityIndex (H'_{loge}), and Pielou's Evenness Index (J') were calculated from quantitative species abundance data by DIVERSE subroutines in PRIMER Version 6.

Multivariate analyses of community composition were undertaken using non-metric multidimensional scaling (nMDS), Analysis of Similarities (ANOSIM) and similarity percentages (SIMPER) procedures using the PRIMER v6.0 statistical software package (Clarke and Gorley, 2006, Clarke and Ainsworth, 1993). Stress values in nMDS provide a measure of goodness of fit for the ordination with values ranging from 0 to 1. Values close to 0 indicate a good fit whereas a stress value greater than 0.3 is no better than arbitrary (Clarke, 1993). The Bray-Curtis distance measure was used to determine similarities between samples, after square-root transformation of abundance data.

One-way ANOSIM was performed to determine whether there were significant differences among groups (a-priori defined) and to compare the similarities in the composition of meiofaunal communities from six locations. Pairwise R values give an absolute measure of how separate groups are on a scale of 0 (indistinguishable) to 1 (all similarities within groups are less than any similarity between groups).

SIMPER analyses were used to determine which species were responsible for compositional differences observed between meiofaunal communities. Clarke and Warwick (1994) stated that as a guideline, species which have a SIMPER ratio greater than 1.3 are likely to be useful for discriminating between groups.

2.3 Results

2.3.1 Distribution of the meiofauna taxa

Sediment meiofaunal communities at Casey were dominated by nematodes, being comprised of 79.8% of nematodes and 20.2% copepods from the total numbers of meiofauna. A total of 58 higher meiofauna taxa comprised of 38 genera of nematodes and 20 families of harpacticoid copepods were identified in 47 samples from six locations. The community compositions were significantly different, (ANOVA, P = 0.003) between locations (Table 2.1). The total mean density of the meiofauna per core varies from 1195 individuals 10 cm^{-2} (Wilkes) to 1601 individuals 10 cm^{-2} (O'Brien Bay-1) with an overall average of 1364 individuals 10 cm^{-2}.

There was significantly difference (ANOVA, $P < 0.05$) between locations in Shannon–Wiener diversity (*H'*), species richness (Margalef's *d*) and evenness (Pielou's *J'*) (Figure 2.2). The most taxa-rich location, Wilkes, comprised 55 taxa (37 genera of nematodes and 18 families of harparcticoid copepods). The location with

the lowest number of taxa was O'Brien Bay-1 (48 taxa; 32 genera of nematodes and 16 families of harparcticoid copepods). The dominant taxa were the nematode genera *Monhystera* and *Daptonema* which were found at all six locations. The most abundant meiofauna taxa at all six locations were *Monhystera* (11.5%), *Daptonema* (8%), *Neochromadora* (6.3%), Tisbidae (4%), *Odonthopora* (3.6%), *Halalaimus* (3.5%) and *Chromadorina* (3.4%), all of which are nematodes except for Tisbidae.

The six study locations could be grouped into two categories: three disturbed locations (Brown Bay Inner, Brown Bay Middle and Wilkes) and three control locations (O'Brien Bay-1, O'Brien Bay-5 and McGrady Cove). The control locations recorded a higher total abundance of meiofauna than the disturbed locations (Figure 2.3a). Based on the SNK results (Table 2.2), the total numbers of copepods taxa were similar in both control and disturbed groups (Figure 2.3c). Mean abundance of copepods were not significantly different between locations. However, the total numbers of nematode taxa were higher in control locations with the highest at O'Brien Bay (Figure 2.3e). The abundance of copepods was significantly less than nematodes in all communities.

Table 2.1: PERMANOVA and ANOVA results of total meiofauna taxa and abundances in spatial study.

Source	DF	MS	F	P
Community composition*				
Location	5	171897	30.25	0.0003
Site (Location)	6	5682.083	0.32	0.9147
Plot (Location X Site)	12	17820.42	1.86	0.0947
RES	24	9582.708		
Nematode Abundance †				
Location	5	174226.5	32.89	0.0003
Site (Location)	6	5297.729	0.29	0.9306
Plot (Location X Site)	12	18290.85	1.88	0.0904
RES	24	9714.854		
Copepod Abundance †				
Location	5	11.4708	7.34	0.0154
Site (Location)	6	1.5625	1.12	0.4069
Plot (Location X Site)	12	1.3958	1.63	0.1477
RES	24	0.8542		
Nematodes Taxa#				
Location	5	0.055	11.31	0.0052
Site (Location)	6	0.0049	0.31	0.9187
Plot (Location X Site)	12	0.0156	2.7	0.0186
RES	24	0.0058		
Copepod taxa †				
Location	5	0.055	11.31	0.0052
Site (Location)	6	0.0049	0.31	0.9187
Plot (Location X Site)	12	0.0156	2.7	0.0186
RES	24	0.0058		

† Data Untransformed * Data Square-root transformed # Data Log transformed

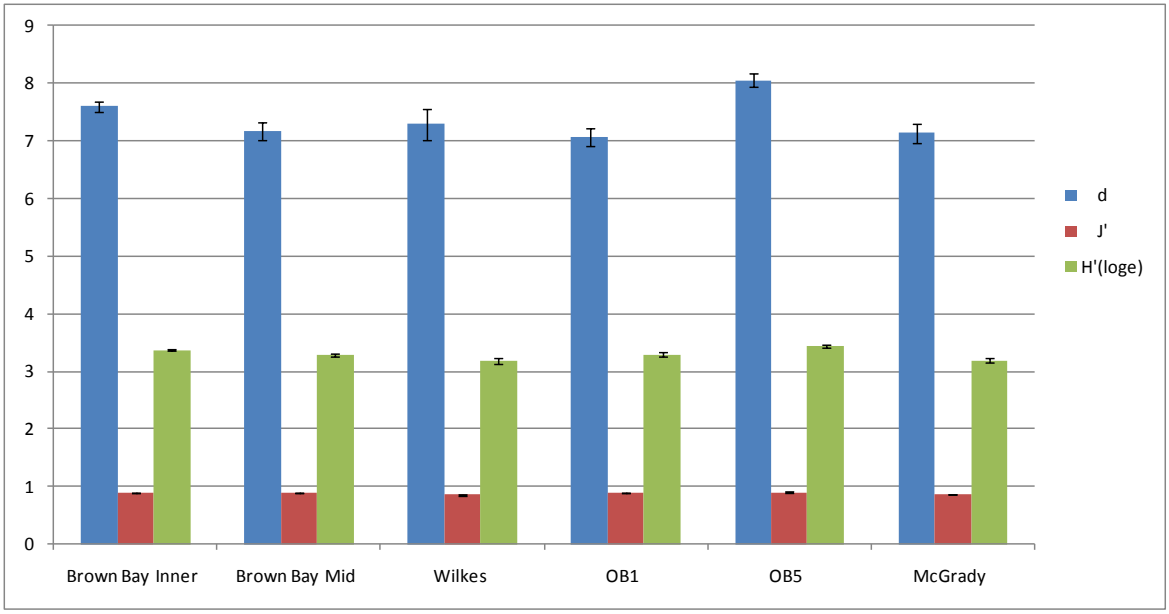

Figure 2.2: Mean abundances (+SE) Shannon–Wiener diversity (*H*' log *e*), species richness (Margalef's *d*) and evenness (Pielou's *J*').

Table 2.2: SNK results of total meiofaunal taxa and abundances in spatial study.

Factors	Location	Site	Plot
Total meiofauna	OB5 = OB1> BB Mid = BB Inner = Wilkes = McGrady Cove	Site 1 = Site 2	Plot 1 = Plot 2
Nematode abundance	OB5 = OB1> BB Mid = BB Inner > McGrady Cove = Wilkes	Site 2 = Site 1	Plot 2 = Plot 1
Nematode taxa	OB5 = BB Inner = OB1 = McGrady Cove= BB Mid = Wilkes	Site 1 = Site 2	Plot 2 = Plot 1
Copepod Abundance	Wilkes = BB Mid = McGrady Cove = OB5 = BB Inner = OB1	Site 1 = Site 2	Plot 1 = Plot 2
Copepod taxa	OB5 = OB1 = BB Inner = McGrady Cove = BB Mid = Wilkes	Site 1 = Site 2	Plot 2 = Plot 1

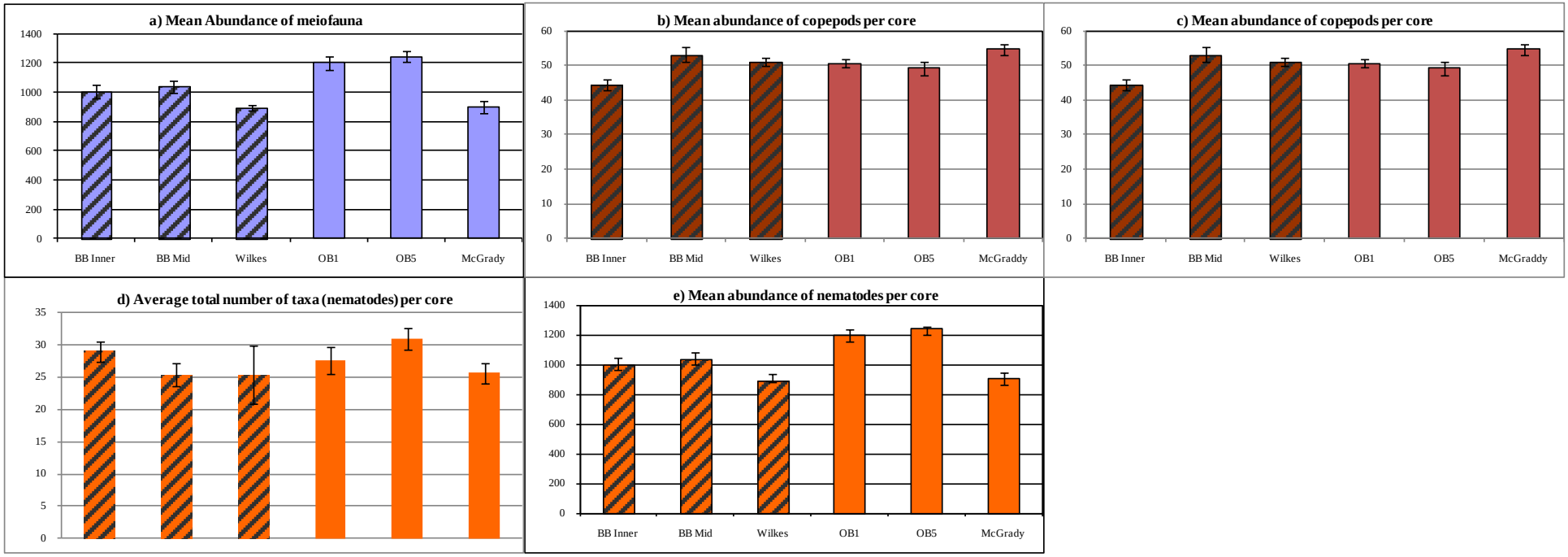

Figure 2.3: Histogram of total number and mean abundance meiofauna found in spatial study at Casey. (BBInner = Brown Bay Inner, BBMid=Brown Bay Middle, OB1=O'Brien Bay-1, OB5=O'Brien Bay-5). Stripe bar represent disturbed locations.

2.3.2 Multivariate analysis of assemblages

Multivariate analysis of assemblage composition revealed significant differences at several spatial scales. The nMDS ordination of all 47 samples in the spatial survey showed distinct grouping of locations in the meiofaunal communities (Figure 2.4a). All locations were significantly different (Table 2.1a) and in the nMDS can be seen to form independent groups except O'Brien Bay-1 and O'Brien Bay-5, where there is some overlap. ANOSIM results (Global R= 0.955, P< 0.01) showed the variation within locations was less than the variation between locations (Table 2.3a and 2.4b).

Pairwise comparisons of O'Brien Bay-1 and O'Brien Bay-5 produced the smallest R values (R=0.713), indicating that these two locations were the least different. These two locations were separated by several kilometres and have a similar composition of meiofaunal communities (stress value 0.17). The meiofaunal communities at O'Brien Bay-1 and O'Brien Bay-5 showed a greater difference between sites within locations than other locations (Table 2.3b, Figure 2.4b).

Browns Bay Inner showed no significant difference between the sites and among plots (Figure 2.4c, Table 2.3b), however there were significant differences between sites at Brown Bay Middle (Figure 2.4d, Table 2.3b). No other locations had significant differences between sites with the location (Figure 2.4e-f, Table 2.3b).

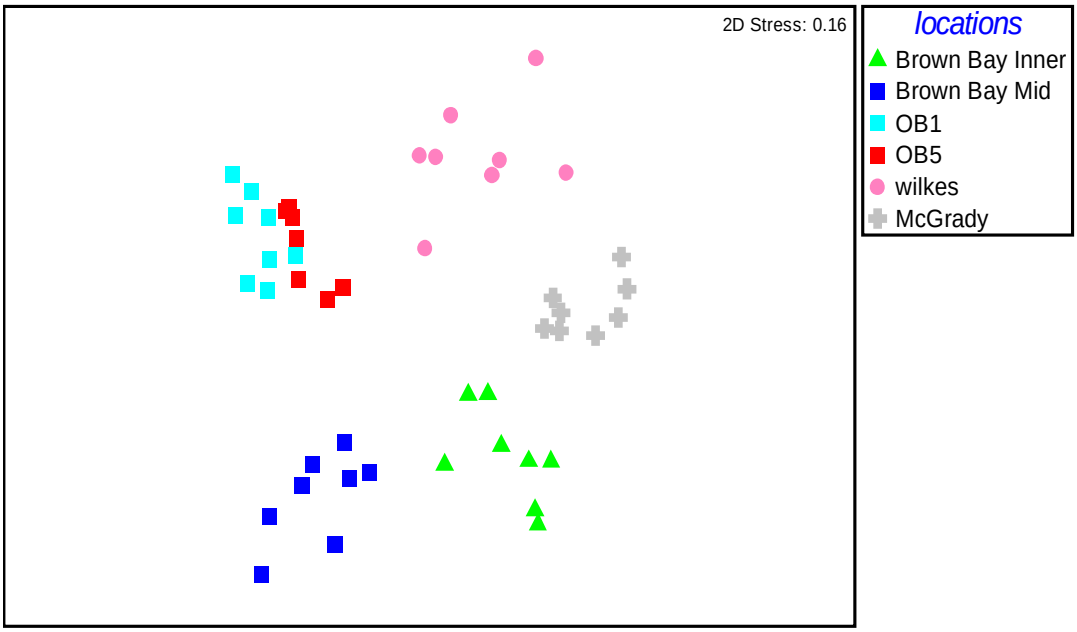

Figure 2.4a: nMDS ordination plots based on square-root transformed meiofaunal abundance data and Bray-Curtis similarities in Casey.

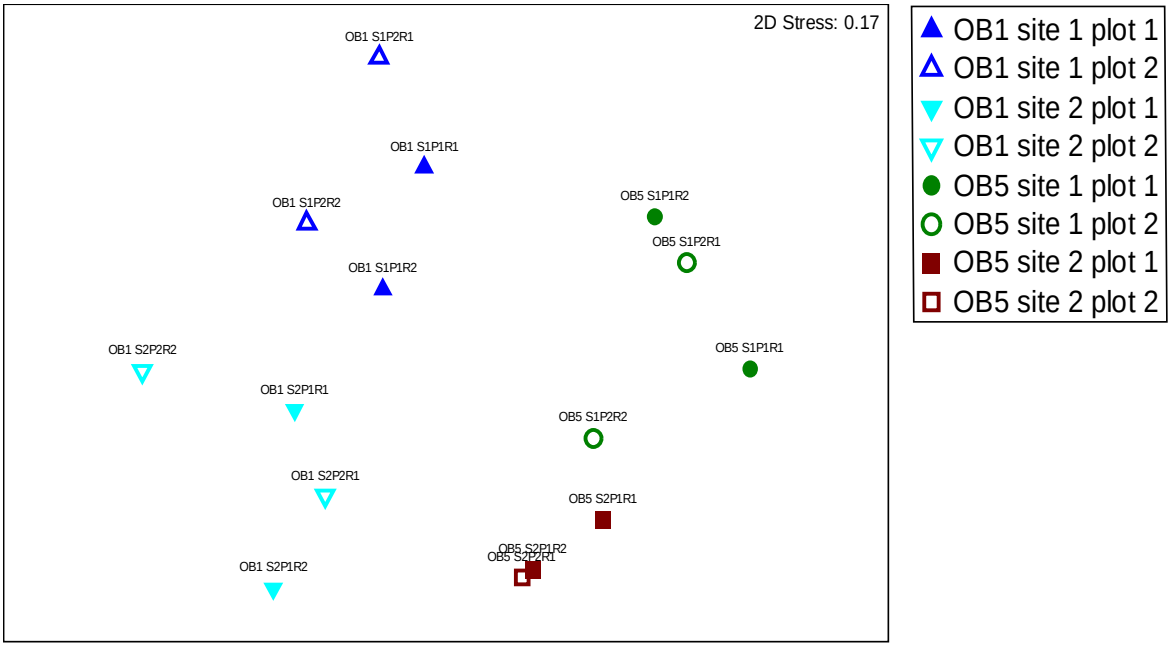

Figure 2.4b: nMDS ordination showing variability in meiofaunal community composition between locations, sites and plots in O'Brien Bay-1 and O'Brien Bay-5.

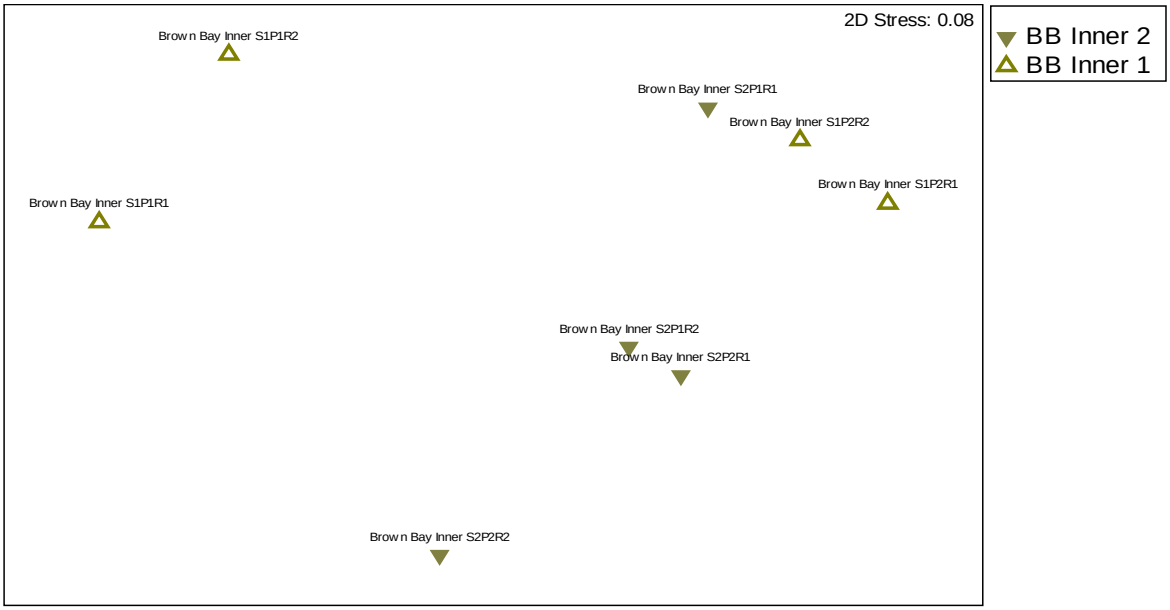

Figure 2.4c: nMDS ordination showing variability in meiofaunal community composition between sites and plots in Brown Bay Inner.

Figure 2.4d: nMDS ordination showing variability in meiofaunal community composition between sites and plots in Brown Bay Middle.

Table 2.3a and 2.3b: One-way ANOSIM results for compositional variation between meiofaunal communities.

Pairwise Tests (significance level used in test)	R-value	P-value (%)
a) Location (Global R = 0.955, Significance level of sample statistic, p: 0.01%)		
Brown Bay Inner, Brown Bay Mid	1	0.02
Brown Bay Inner, O'Brien-1	1	0.02
Brown Bay Inner, O'Brien-5	1	0.02
Brown Bay Inner, Wilkes	0.987	0.02
Brown Bay Inner, McGrady Cove	1	0.02
Brown Bay Mid, O'Brien-1	1	0.02
Brown Bay Mid, O'Brien-5	1	0.02
Brown Bay Mid, Wilkes	0.998	0.02
Brown Bay Mid, McGrady Cove	1	0.02
Wilkes, McGrady Cove	0.991	0.02
O'Brien-1, O'Brien-5	**0.713**	0.02
O'Brien-1, Wilkes	0.98	0.02
O'Brien-1, McGrady Cove	1	0.02
O'Brien-5, Wilkes	0.948	0.02
O'Brien-5, McGrady Cove	1	0.02
b) Site (Global R= 0.895, significance level of sample statistic, p: 0.01%)		
BB Inner, Site 1 vs. Site 2	0.115	20
BB Mid 1, Site 1 vs. Site 2	**0.354**	**2.9**
Wilkes, Site 1 vs. Site 2	-0.094	65.7
O'Brien-1, Site 1 vs. Site 2	**0.594**	**2.9**
O'Brien-5, Site 1 vs. Site 2	0.611	5.7
McGrady Cove, Site 1 vs. Site 2	0.229	5.7

Figure 2.4e: nMDS ordination showing variability in meiofaunal community composition between sites and plots in Wilkes.

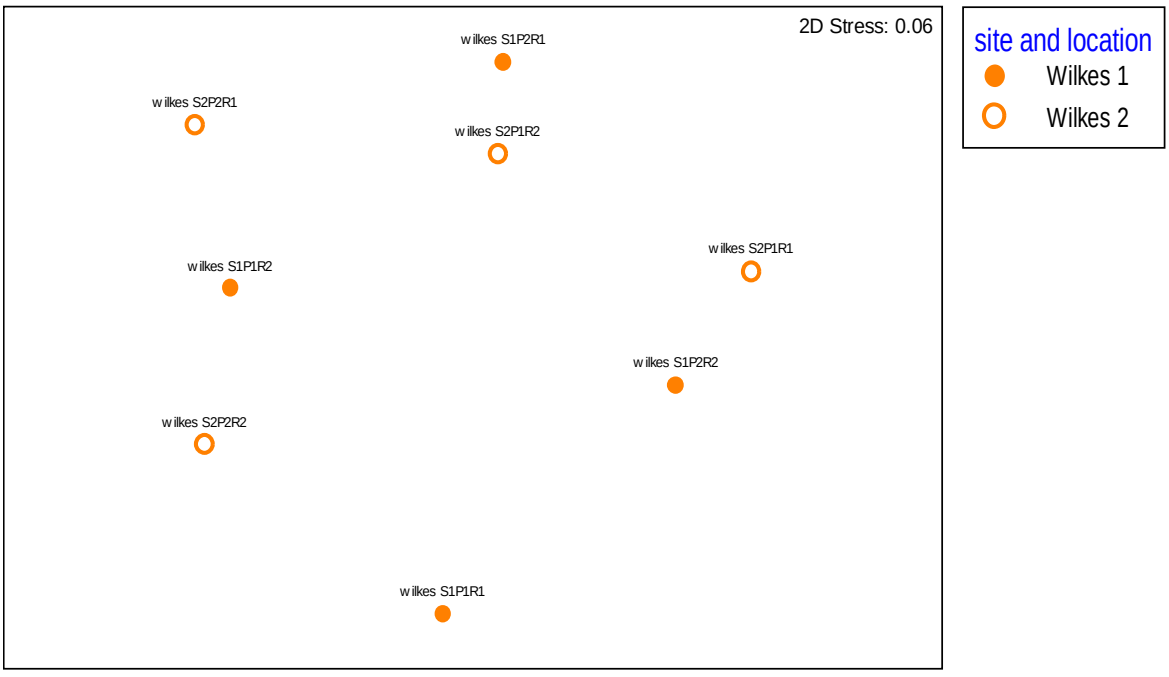

Figure 2.4f: nMDS ordination showing variability in meiofaunal community composition between sites and plots in McGrady Cove.

2.3.3 Compositional differences between meiofaunal communities

Similarity percentage analysis (SIMPER) was used to determine the taxa contributing to the observed differences between and within locations. The SIMPER analysis (Table 2.4) showed that *Monhystera*, *Daptonema* and *Neochromadora* are among the taxa that contributed the most to dissimilarity between locations (Figure 2.4). The genera *Monhystera* and *Neochromadora* were found at all six study locations, including the impacted and control locations and could be considered to be tolerant of a range of conditions (Table 2.4). Three genera of nematodes, *Sabatiera*, *Theristus* and *Spirobolbolaimus*, were considered important only in Brown Bay Inner due to their high contribution to total abundance. In contrast, *Leptolaimus*, Huntemanniidae and Cletodidae were found to be unique taxa to Wilkes. Two taxa, *Promonhystera* (nematode) and Paramesochridae (harpaticoid copepod), were only found in the disturbed locations of Brown Bay Inner, Brown Bay Middle and Wilkes. Six genera were found only at control locations O'Brien Bay-1 and O'Brien Bay-5 and McGrady Cove: *Megadesmolaimus*, *Metalinhomoeus*, *Molgolaimus* and Ancorabolidae from O' Brien Bay; and Miraciidae and Canuellidae, harpaticoid copepods, were important genera at McGrady Cove.

Table 2.4: SIMPER analysis showing family/genera ranked according to average Bray-Curtis similarity within groups. The list of genera was limited to a cumulative percentage dissimilarity of 70%. Shaded columns represent disturbed locations.

Location (Ave. similarity)	BB Inner (80.88)		BB mid (80.61)		Wilkes (74.56)		O'Brien (81.44)		McGrady Cove (84.34)	
Taxa	Ave	Contrib %	Ave	Contrib %	Ave	Contrib %	Ave	Contrib %	Ave	Contrib%
Monhystera	4.8	5.84	5.88	7.72	5.44	7.56	5.13	6.43	5.5	7.04
Neochromadora	3.78	4.78	4.72	6.05	4.45	6.36	2.94	3.16	4.25	5.12
Linhomoeus	3.8	4.77					2.01	2.31		
Daptonema	4.05	4.73	2.1	2.06	4.95	7.01	4.53	5.61	5.54	7.17
Odonthopora	3.91	4.69	2.9	3.58	4.04	5.12				
Paralinhomoes	3.19	3.81	3.5	4.51	3.78	5.45				
Tisbidae	2.99	3.65	2.58	2.83			2.83	3.33	3.43	4.17
Chromadorina	2.88	3.29	3.81	4.76	2.7	3.33	2.08	2.16	3.06	3.62
Promonhystera	2.6	3.23	3.05	3.81					1.99	2.33
Chromadora	2.69	3.09	2.15	2.13			3.35	4.15	2.49	2.83
Paramesochridae	2.29	2.65	2.71	3.5	1.78	2.42	2.61	3.01		
Draconema	2.33	2.65								
Ascolaimus	2.37	2.64	2.35	2.61			3.25	3.89		
Chromadorella	2.3	2.58	2.78	3.46			2.64	3.27	2.36	2.51
Desmodora	2.2	2.49								
Wieseria	2.17	2.45							2.73	3.29
Bolbolaimus	2.22	2.33	1.97	2.28			2.64	3.06		
Sabatiera	1.97	2.24								
Theristus	2.13	2.21								
Spirobolbolaimus	2.09	2.2								
Southerneilla	2.02	2.16							2.27	2.35
Sphaerolaimus	1.94	2.09							3.16	3.87
Gammanema			2.54	3.13					2.06	2.42
Ectinosomatidae			2.34	3.03	2.47	3.55	2.21	2.65	2.57	3.29
Paracanthonchus			2.3	2.64	2.62	2.37				
Paramonhystera			2.33	2.49						
Canthocamptidae			1.96	2.35					1.76	2.21
Pierrickia			1.93	2.32						
Ixonema			4.01	5.08						
Halalaimus					3.4	4.88	2.58	3.06	4.87	6.2
Leptolaimus					3.38	4.84				
Chromadorina					2.7	3.33			3.06	3.62
Dactylopusiidae					2.27	3.13	1.97	2.14		
Dichromadora					2.04	2.68	2.49	2.88		
Huntemanniidae					1.86	2.6				
Aegisthidae					1.61	2.33			1.92	2.23
Microlaimus					2.04	2.1	2.43	2.92		
Zosimidae					1.43	1.92	1.86	1.99	2.34	2.96
Chromadorita					1.69	1.84			2.66	2.54
Cletodidae					1.26	1.75				
Megadesmolaimus							4.23	5.13		
Metalinhomoeus							3.8	4.78		
Molgolaimus							2.52	2.97		
Ancorabolidae							1.78	2.09		
Miraciidae									2.11	2.64
Canuellidae									2.1	2.58
Total taxa (Nematodes:Copepod)	22 (20:2)		20 (16:4)		20 (13:7)		21 (15:6)		21 (13:7)	

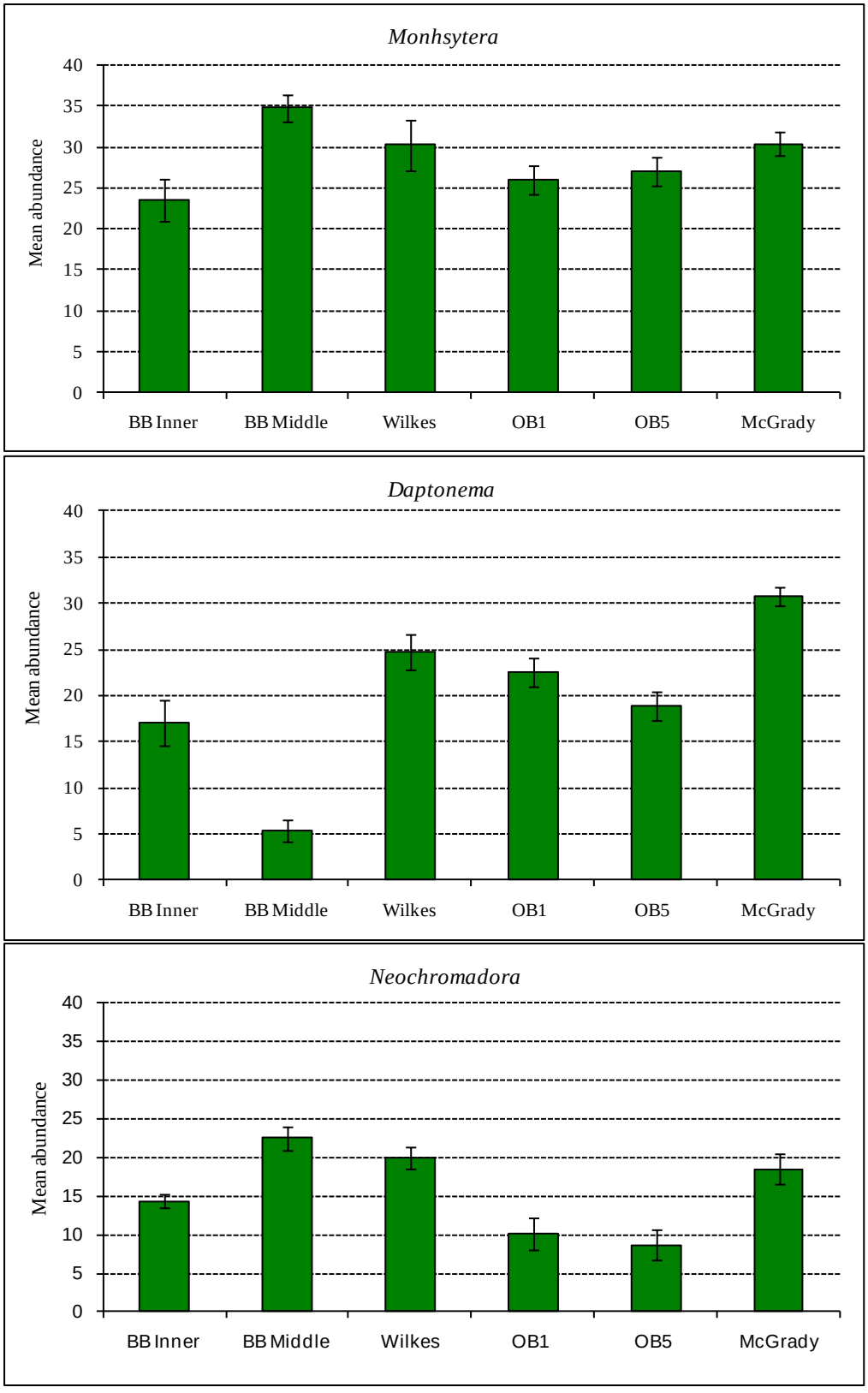

Figure 2.5: Mean abundances of genera which have the highest contribution in all locations.

The SIMPER analysis of the meiofaunal communities shows that the average dissimilarity of O'Brien Bay-1 and O'Brien Bay-5 was very low (20.74%) and was due to 43 taxa (26 genera of nematodes and 17 families of copepods) out of the 58 recorded taxa, the most dominant of which were *Sphaerolaimus* (3.83%), Ameiridae (3.69%) and Rometidae (3.16%). As O'Brien Bay-1 and O'Brien Bay-5 were the most similar locations; their distance is less than 200m., they were combined into one group in a SIMPER analysis comparison with other locations. A total of 46 taxa (35 genera of nematodes and 11 families of copepods) contributed to the average dissimilarity (38.76%) of the groups Brown Bay Inner and O'Brien Bay, represented mainly by *Megadesmolaimus* (5.91 %), *Promonhystera* (3.63%), *Metalinhomoeus* (3.62%) and *Wieseria* (3.03%). In contrast, a total of 44 taxa (31 genera of nematodes and 13 families of copepods) contributed to the average dissimilarity (36.88 %) of the groups Brown Bay Middle and O'Brien Bay, which were *Promonhystera* (4.5%), *Ixonema* (4.29%), *Megadesmolaimus* (3.9%), *Molgolaimus* (3.7%) and *Daptonema* (3.63%). *Megadesmolaimus* was more abundant in Brown Bay Inner than Brown Bay Middle, whereas *Promonhystera* showed the opposite pattern.

The spatial variation of meiofaunal communities at Casey was significant at three scales: between locations (1000's of meters) between sites within locations (100's meters) and between plots within sites (10's of meters). This study shows that the taxa were most different at the largest scale (locations). These were supported by variance component estimates for all scales (Table 2.5). *Neochromadora*, *Pierrickia*, *Sabatieria* and *Spirobolbolaimus* were not significantly different at the largest scale. Meanwhile, *Daptonema*, *Neochromadora*, *Megadesmolaimus*, *Metalinhomoeus*, *Molgolaimus*, *Paralinhomoes*, Ancorabolidae and Canuellidae were significantly

different at medium scales. The taxa that were significantly different at the finest scale were *Daptonema, Ixonema, Neochromadora, Megadesmolaimus, Molgolaimus, Monhystera, Odontophora, Paramonhsytera, Pirrickia, Theristus*, Huntemanniidae, Miraciidae and Zosimidae.

Table 2.5: Summary of significant results of ANOVA tests and post-hoc comparisons by SNK tests for taxa at different scales and estimates of variance components for three factor-nested design.

Taxa	**Location**	**Site**	**Plot**	**Variance estimates %**			
				Location	**Site**	**Plot**	**Residual**
Daptonema	McGrady Cove = Wilkes = OB1 = OB5 = BBInner > BBMid	BBInner*	BBInnerS1*	74.3	1.8	0.0	23.9
Halalaimus	McGrady Cove > Wilkes > OB5 = OB1 > BBMid = BBInner	NS	NS	90.8	0.0	1.4	7.8
Ixonema	BBMid > Wilkes = OB5 =OB1=McGrady Cove=BBInner	NS	BBMidS1** BBMidS2** WilkesS1**	90.3	0.0	7.4	2.3
Leptolaimus	Wilkes > BBInner > BBMid = OB5 = OB1 = McGrady Cove	NS	NS	78.7	0.0	0.0	21.3
Neochromadora	NS	OB1* OB5*	McGrady CoveS2*	49.4	16.5	7.0	27.1
Megadesmolaimus	OB1 = OB5 > BBMid = Wilkes = McGrady Cove = BBInner	OB1* OB5**	WilkesS2*	82.1	13.6	1.0	3.3
Metalinhomoeus	OB1 = OB5 > Wilkes = BBMid = BBInner = McGrady Cove	OB5* Wilkes**	NS	65.9	13.9	0.0	20.2
Molgolaimus	NS	Wilkes*	OB1S1** WilkesS2**	59.8	3.2	18.6	18.4
Monhystera	BBMid >all others	NS	BBInnerS1* WilkesS2*	24.6	0.0	32.3	43.1
Odontophora	Wilkes = BBInner > BBMid > OB1 = McGrady Cove = OB5	NS	WilkesS1** WilkesS2**	55.0	0.0	24.7	20.3
Paralinhomoes	BBMid > OB5 = all others	BBInner* OB*	NS	68.9	12.9	0.0	18.2
Paramonhsytera	BBMid > OB5 = all others	NS	BBMidS1* OB5S1*	65.1	0.0	22.9	11.9
Pierrickia	NS	BBInner*	BBMidS1*	44.6	27.9	3.9	23.6
Sabatieria	NS	NS	NS	28.2	0.0	0.0	71.8
Spirobolbolaimus	NS	NS	BBInnerS1**	50.6	0.0	21.0	28.4
Theristus	BBInner > Wilkes =all others	NS	BBInnerS1* WilkesS2**	37.0	0.0	17.6	45.4
Ancorabolidae	OB5 > OB1 = BBMid = McGrady Cove > Wilkes = BBInner	BBMid*	NS	71.7	4.6	0.0	23.8
Canuellidae	Wilkes = McGrady Cove = OB5 = OB1 = BBMid = BBInner	OB5*	NS	35.5	24.7	0.0	39.9
Cletodidae	Wilkes > McGrady Cove > OB5 = OB1 = BBMid = BBInner	NS	NS	56.7	0.0	0.0	43.3
Huntemanniidae	OB5 = Wilkes = BBInner = BBMid = McGrady Cove = OB1	NS	BBInnerS1* BBMidS2**	24.5	6.5	20.3	48.7
Miraciidae	McGrady Cove = BBMid = BBInner = Wilkes = OB5 = OB1	NS	BBInnerS2**	56.7	0.0	13.2	30.1
Zosimidae	McGrady Cove = OB1 > BBInner = BBMid = OB5 = Wilkes	NS	OB1S2*	71.5	0.0	5.1	23.5

2.4 DISCUSSION

The macrobenthos and microbenthic flora have been the focus of several studies in coastal areas at Casey Station (Stark et al., 2003b, Cunningham et al., 2003a, Stark, 2000) but the meiofauna are an important component of this benthic ecosystem about which nothing is known. In general only limited studies have been done on the benthic meiofauna in Antarctica e.g. (de Skowronski and Corbisier, 2002, De Mesel et al., 2006).This study provides the first general picture of the spatial distribution and structure of the meiofaunal communities and their variability at Casey, Antarctica.

2.4.1 General meiofaunal distribution

The results clearly demonstrate that nematodes are much more abundant by comparison to copepod in the coastal areas at Casey. Similar results were obtained by other studies. Whereby, meiofaunal communities are dominated by nematodes, having at least 80% of nematode in the total abundance (Van Holsbeke, 1988, Herman and Dahms, 1992, Fabiano and Danovaro, 1999). For example, it was reported that in Martel Inlet, King George Island, Antarctica, at a depth of 15 m, the meiofauna taxa was dominated by nematodes (> 60%), followed by copepods, nauplii and polychaetes (de Skowronski and Corbisier, 2002), while in the deep waters of Halley Bay, Weddell Sea (500-1000 m) a total of only 16 meiofauna taxa was recorded, with nematodes comprising 90% of the total abundance (Herman and Dahms, 1992). The average total mean density of the meiofauna at Casey (1364 individuals per 10 cm^2)

were higher than those observed in Ross Sea, which were 1191 individuals per 10 cm^2 (Fabiano and Danovaro, 1999) but lower than in Halley Bay (1677 individuals per 10 cm^2), in Weddell Sea (Herman and Dahms, 1992). The differences between these studies were mainly caused by differences in water depth where samples were taken, since both studies (Ross Sea and Weddell Sea) were done at depth more than 500 m. Apart of influences by water depth, equipment and collection method used may also explain the variability in results. For example, differences in densities were visible between organism sampled from the uppermost 3 cm of substrate using hand-corer (Veit-Köhler et al., 2008) and sample taken at 5 cm using Van Veen grab (Kotwicki et al., 2004) at Kongsfjorden. Although the total density showed variation, all studies showed increases in nematode abundance was paralleled with a decrease in copepod abundance (Kotwicki et al., 2004, Veit-Köhler et al., 2008). Table 2.6 summarise the density of meiofauna found several locations around the world. In this current study, the total number of meiofauna taxa recorded at Casey was 58, with 38 nematode genera and 20 copepod families. The results contained in this study are similar to those of Lee et al. (2001a) from the deep continental shelf of Kapp Norvegia in the eastern Weddell Sea, where a total of 38 nematode genera were found.

Table 2.6: Comparison of meiofaunal communities around the world (modified from Herman and Dahms (1992)).

Reference	**Area**	**Depth range (m)**	**Density range (N.ind cm^{-2})**
Alongi and Pichon (1988)	Pacific- Coral Sea	298 - 1610	19-170
Bouvy and Soyer (1989)	Kerguelen Island	sea-level	15,000
Bovee and Soyer (1975)	Kerguelen Island -muddy site -sand/gravel -spicule-mat	 4-193 4-167 26-52	 121 2,580 421
Bovee et al. (1990)	Gulf of Lion	500-2,500	150-1,500
Dinet and Vivier (1977)	Atlantic - Golfe de Gascogne	2,000 2,000-3,000 4,100-4,700	539 407-500 94-305
Shirayama (1984)	Western Pacific - deep-sea	2,100-8,300	37 - 1,315
Snider et al. (1984)	Central North Pacific	5,800	102
Pfannkuche and Thiel (1987)	NE-Svalbard Shelf Nansen Basin	226-320 3,920	1,143- 3,439 348
Herman and Dahms (1992)	Halley Bay, Weddell Sea	340 1,960	120-3,800
Fabiano and Danovaro (1999)	Ross Sea	439-567	192-1192
Kotwicki et al.(2004)	Kongsfjorden, Svalbard	44-350	1.1-2098
Veit-Köhler et al.(2008)	Kongsfjorden, Svalbard	20	1220-1613
Present study	Casey-subtidal	12-20	1195-1601

2.4.2 Spatial variation

Spatial variation of meiofaunal communities at Casey Station was observed between locations (1000's of meters) between sites within the location (100's meters) and between plots within site (10's of meters). Significant differences in meiofaunal communities were strongest found at the largest scale, between locations.

Nematodes (genus) and copepod (family) taxa appeared to be very patchy and showed greatest significant variation at the scale of locations (large) than plots (fine). Comparisons among locations (large scale) and plots within locations (fine scale) can be done using lower (genus level) taxonomic resolution for nematodes and higher (family level) taxonomic resolution for copepods. Similarly, Herman & Heip (1988) and Warwick (1988a) found family level data of meiofauna appropriate in explaining pollution gradients and recommended family level identification from a practical point of view. Veiga et al. (2009) studied the meiofaunal community structure at higher taxonomic levels and distinguished the Prestige oil spill impacted site from a non-polluted reference site. However, Heip et al. (1988) observed significant loss of information for nematodes only at the level of sub-orders whereas for copepods, it occurred at family level. In a study on the macrofaunal soft sediment assemblages by Stark et al. (2003b), significant differences were observed at all scale, and most of the variation was associated with the largest and the smallest scale. Although, the nMDS ordination analysis showed that there was a very distinctive pattern between control and disturbed locations. Significant differences within location were only observed in

Brown Bay Middle and O'Brien Bay, while the other locations showed no differences.

Higher abundances of meiofauna were recorded in control locations (O'Brien Bay, except for McGrady Cove) than disturbed locations (Brown Bay and Wilkes). The highest numbers of taxa were present at Wilkes (55 taxa) and the lowest numbers of taxa at O'Brien Bay-1 (48 taxa). Differences in number of taxa between both locations were mainly caused by the geographical factor, physical differences and other anthropogenic influences since O'Brien Bay is a location furthers from human activities sites. While, Wilkes is categorizes as disturbed location and located offshore from a large disused tip. Impacts associated with the tip site in Brown Bay and abandoned Wilkes dump site could increase organic input, heavy metals and sedimentation (Stark, 2000), and these could contribute to the differences between control and disturbed locations.

It was first proposed by Raffaelli and Mason (1981) of the use of the nematode and copepod ratio as tool for biomonitoring. The validity of this proposal has been argued (Coull et al., 1981) and accepted with modification by many (Moreno et al., 2008b, Lee et al., 2001b). The ratio of nematodes to copepods of at least 4:1, which is similar to other regions of the world, e.g. the Mediterranean (De Leonardis et al., 2008, Moreno et al., 2008b), as well as other areas in Ross Sea, Antarctica (Fabiano and Danovaro, 1999). However, both disturbed and controlled sites around Casey station had higher numbers of nematodes by comparison to copepods. Hence, the usage of the ratio solely is doubt as there are difficulties in separating the effects of pollution

on the ratio from the effects of other environmental variables (Lambshead, 1984) as Stark et al (2003a), showed that the significant differences in chemical and environmental properties between both disturbed and controlled location around Casey station. Although numbers of nematodes were higher in all locations, the abundance and taxa distribution were also variable between control and disturbed locations.

There were significant differences in the meiofaunal community between locations. Wilkes, a potentially disturbed location, was most similar to the control locations. One possible reason for this is that the Wilkes waste dump site is much older than the Casey site and has not been disturbed recently and does not have a melt stream running through it. Thus, this location may have recovered from pollution impacts or may never have been as strongly impacted as Brown Bay. Wilkes also recorded a high percentage of copepods by comparison to other disturbed sites which was similar to the control sites of McGrady Cove and O'Brien Bay, suggesting that the benthic environment in Wilkes is a suitable habitat for pollutant sensitive organisms. This is supported by the findings of Warwick, (1986) who suggested that copepods are in generally more sensitive to the effects of pollution than nematodes. Similarly, Stark et al., (2003b) found that the macrofaunal communities at Wilkes were similar to those of the control sites (O'Brien Bay) in some ways but in other ways resembled an impacted location, suggesting that perhaps it was impacted at some point in its past since human occupation.

Data obtained showed that the differences between locations were greater than within locations. However, within Brown Bay, the differences between the Inner and Middle locations were as great as between locations separated by many kilometres (as indicated by high R-values: R=1) even though they are only several hundred metres apart. This is possibly due to the presence of the genus *Ixonema* which contributed up to 4.0% in Brown Bay Middle but were not found in Brown Bay Inner. In addition, the genera *Gammanema* and *Paramonhystera* were only present in Brown Bay Middle. The position of Brown Bay Inner and Brown Bay Middle relative to the tip site may also influence the meiofauna distribution, as Brown Bay Inner is located nearer to the tip site (15 m) than Brown Bay Middle (300 m off shore). This finding is in agreement with those of (Stark, 2000) who reported a variation in macrobenthos abundance between two locations within Brown Bay, which were affected by the pollution from the garbage tip. As for O'Brien Bay-1 and O'Brien Bay-5, both locations showed great similarity as the R-values were only 0.713, where the contribution of all species were about similar. In addition, both locations are located in the same embayment which has not been influenced by any station activities.

Differences in environmental conditions due to pollution are likely to have contributed to the differences between locations in the meiofaunal communities. For example, the genus *Sabatieria* was found in Brown Bay Inner, which was known to be contaminated from human activities. This genus is also found in areas, which has fine sediment substrate (Vanhove et al., 1998) such as Brown Bay. Other studies have suggested that this genus has a high tolerance to pollution or disturbance (Somerfield et al., 1994) and survives low oxygen concentrations that are unsuitable for most other nematodes genus. Furthermore, the genera *Paramonhsytera* and *Theristus*, which

were found to be tolerant to metal pollution by Gyedu-Ababio et al. (1999), were found be important taxa in Brown Bay (Table 2.7). Gyedu-Ababio et al. (1999) also suggest that *Paramonhsytera* and *Theristus* as a possible indicator genus of pollution. Schratzberger et al. (2002) found that *Leptolaimus* was sensitive to high levels of disturbances and *Daptonema* was considered as a pollutant indicator. Similar observation on *Daptonema* as a non-selective feeder was also reported from Signy Island (Vanhove et al., 1998) and Kapp Norvegia (Vanhove et al., 1999). Both genera were recorded as important taxa in the disturbed locations at Casey.

Table 2.7: Summary of important, tolerant and indicator taxa in Casey, Antarctica.

Site	**Important taxa**	**Tolerant taxa**	**Indicator taxa**
O'Brien Bay (1 and 5)	*Megadesmolaimus,* *Metalinhomoeus* *Molgolaimus* Ancorabolidae	*Monhystera* *Neochromadora* *Daptonema* *Chromadorina*	Sensitive: *Halalaimus* Zosimidae
McGrady Cove	Miraciidae Canuellidae		
Brown Bay Inner	*Sabatieria* *Theristus* *Spirobolbolaimus*		Pollutant: *Odontophora* *Paralinhomoes*
Brown Bay Middle	*Paramonhsytera* *Pierrickia* *Ixonema*		
Wilkes	*Leptolaimus* Huntemanniidae Cletodidae		

The genera *Monhystera* and *Daptonema* were found at all six locations. *Monhystera* has a previously reported worldwide distribution, from the polar to the tropical regions (Gerlach and Schrage, 1971, Vanhove et al., 1998, Vanhove et al., 1999, Vanaverbeke et al., 1997, Lee et al., 2001a) and has been found abundantly in

organically enriched and polluted sediments (Lorenzen et al., 1987) and is known as a colonizer genus and is considered an indicator of pollution (Veiga et al., 2009). This characteristic explains the widespread distribution of the genus thus suggesting that this genus is a tolerant species. Nematodes of the genus *Neochromadora* and *Chromadorina* are also considered to be tolerant species due to their presence in all locations. The genus *Daptonema* is also typically found in muddy and heavily polluted sediments (Moreno et al., 2008a) and has been proposed to be representative of a community that is well adapted to disturbed conditions (Brandt et al., 2007).

Finding from this study suggest that by studying meiofaunal assemblages at fine to largest scale and identifying taxa at higher taxonomic resolution level could distinguished the potential impacted sites around Casey region. This study also supports the suggestion by Stark et al (2003a) that survey identification to medium levels of resolution (family, suborder) could be done to reduce samples sorting, time and lessen the cost. Information baseline data on opportunistic, important and sensitive taxa could be gathered from this study for future research monitoring.

3.0: THE INFLUENCE OF ENVIRONMENTAL VARIABLES ON MEIOFAUNAL COMMUNITIES AT CASEY

3.1 Introduction

The relationships between benthic macrofauna communities from polar waters and environmental factors have been studied extensively for many years. In contrast, studies on meiofaunal (small metazoans passing through a 500 μm mesh sieve but retained on a sieve 32 μm) communities and the environmental factors affecting them are still relatively poorly known, especially in Antarctica. However, the importance of meiofauna has been emphasized in many studies (Gerlach, 1971, Alheit and Scheibel, 1982). These studies have concluded that meiofaunal communities are responsible for stimulating microbial activity in the sediment (de Skowronski and Corbisier, 2002, De Mesel et al., 2006, Mamilov and Dilly, 2002), they themself are food for the macrofauna (Coull, 1990, Pihl and Rosenberg, 1984, Leduc and Probert, 2009) they can be a potential bioindicator of environmental impacts (Moore and Bett, 1989, Moreno et al., 2008, Sutherland et al., 2007, Kennedy and Jacoby, 1999, Gyedu-Ababio et al., 1999, Schratzberger et al., 2001) and their production is greater than that of macrofaunal communities.

The abundance of meiofauna is largely influenced by environmental factors such as nutrients, salinity, sediment grain size and physical and chemical sediment disturbances (sedimentation and pollution). These factors however, vary from large

scales (kilometres) to small scales (meters) (Alves et al., 2009). At larger scales (km), the distribution of meiofauna is commonly related to abiotic gradients in sediment composition and granulometry, salinity, temperature changes and tidal or wave actions (Alves et al., 2009, Soetaert et al., 1995). At smaller scales (meters), distributions are determined by the availability of food sources (Franco, 2007, de Skowronski and Corbisier, 2002), predation (Hoste et al., 2007), reproductive behaviour (Alves et al., 2009, Soetaert et al., 1995) and disturbance (Austen and Widdicombe, 2006, Gallucci et al., 2008, Lee et al., 2001a) .

Although it is not yet clear which factors are most important for meiofauna distribution in polar areas, de Skowronski & Corbisier (2002) emphasized that food availability in the substratum and sediment grain size could be the most important factors determining the distribution of the meiofauna in Antarctic seas. For example, the biovolume of harpacticoid copepods was found to be related to food supply, in terms of carbon and nitrogen availability, produced by benthic and pelagic primary production in Potter Cove, Antarctica (Veit-Kohler, 2005). In addition, Veit-Kohler (2005) also found that the abundance of harpacticoids had a positive relationship with total organic matter. Effects of sediment grain size on meiofaunal distributions have been observed in other studies (Alves et al., 2009). For example, in a study of the intertidal sand substrata around Iceland, a positive relationship was found between sediment properties (utilizable organic matter, fine sand fraction) and meiofaunal abundance (Delgado et al., 2009). Although the relationship between meiofauna and sediment grain type has been emphasized in many studies, Kotwicki et al. (2004) observed that in the intertidal areas in Kongsfjorden, Arctic, the lack of sediment

stability and permanent mineral sedimentation processes also appeared to be an influencing factor on the distribution of meiofauna.

In addition to sediment grain size and food availability, heavy metal contamination has also been found to be a major disturbance on soft sediment assemblages in Antarctica (Stark et al., 2003b, Cunningham et al., 2005). For example at Casey Station, Antarctica, heavy metal and hydrocarbon contamination has been found around two abandoned waste dumps (Stark et al., 2006, Stark, 2000, Cunningham et al., 2003b, Stark et al., 2003b). There are significant differences here between the soft sediment infaunal assemblages of the impacted sites and the nearby control areas (Stark, 2000, Stark et al., 2003b). Stark et al. (2003b), demonstrated that the impacted locations around Casey Station had lower diversity and lower species richness. They concluded that differences in soft-sediment assemblages between control and potentially impacted locations were closely correlated with concentrations of heavy metals in the sediments. It has been previously shown that an increase in metal concentration can cause a decrease in nematode diversity (Tietjen, 1980). In addition, Somerfield et al (1994), have also shown that the nematode community structure in the Fal Estuary, England changed consistently with increasing sediment metal concentration, while changes in copepod abundance were more random.

To date there are limited studies examining the influence of environmental variables on meiofaunal assemblages in Antarctica. In this Chapter, the following questions are addressed:

- Are the composition, abundance and spatial variation of meiofaunal communities at Casey related to environmental variables such as metals, grain size and organic carbon?

3.2 Materials and Methods

Sampling design: Sediment samples were collected by divers using small core tubes (16 mm diameter) from six locations including three control locations and three disturbed locations, adjacent to waste disposal sites. Sampling followed a hierarchical nested design at three spatial scales, from ~10 meters, ~100 metres to kilometres, to determine the spatial patterns of community composition and abundance. Two disturbed locations were situated along a gradient of pollution within Brown Bay (Inner and Middle), the other represented by a single, potentially impacted area near Wilkes (Figure 3.1). Within each location there were two sites (~ 100 m apart) and within each site there were two plots (~10 m apart). Within each plot (1 m diameter), two replicate cores were taken.

3.2.1 Location Description

Casey Station

Casey Station is situated at 66^{o}17’ S, 110 ^{o} 32’E on Bailey Peninsula in the Windmill Islands, Antarctica (Figure 3.1). The shallow (<50 m) benthic marine environment in

the near shore region at Casey is very heterogeneous (Stark, 2003). In this study, the locations selected around Casey Station were Brown Bay (Middle and Inner), Wilkes, O'Brien Bay (1 and 5) and McGrady Cove.

Brown Bay

Brown Bay is a small embayment at the southern end of Newcomb Bay with a maximum depth of approximately 20 m and with rocky sides grading to a muddy bottom (Cunningham et al., 2003b). Brown Bay is typically ice free for 1-2 months a year, usually in January and February, and the ice does not usually break out in winter. Brown Bay is situated adjacent to the abandoned Casey waste dump, which is on the foreshore of the bay, at the base of Thala Valley (Stark et al., 2003c). Samples were taken from 2 locations within Brown Bay: Brown Bay Inner was 50 m from the waste dump directly in front of the point where summer melt water from the valley enters the bay (Stark et al., 2006) and the water depth was 7 m. Brown Bay Middle was 200 m from the waste dump and ranges from 12 to 15 m in depth. .

Wilkes

The first Australian station in the area, Wilkes was built in 1957 on Clark Peninsula on the shore of Newcomb Bay but was abandoned in 1969 (Cunningham et al., 2005). The Wilkes waste dump site is on the foreshore of a small embayment on the northern side of Newcomb Bay. Samples were collected from approximately 100 m directly in front of the waste dump.

O’Brien Bay

O’Brien is a large bay situated several kilometres south of Casey Station. This site is clean and unlikely to have been contaminated by human activities. Therefore, the area was selected as a reference location. The benthic habitat consists of rocky bottoms, boulders, cobbles and gravel overlain by patches of sediment (Stark et al., 2003a). Samples were taken in 15-20 m water depth.

McGrady Cove

McGrady Cove is a small embayment situated in the north-west corner of Newcomb Bay. The northern shore consists of low ice cliffs (up to 5m) and the southern side of the bay is a rocky slope. No human activities are known to have occurred in the bay and it was intended as a reference location within Newcomb Bay. Samples were collected on the northern side of the bay in 12 – 15 m depth.

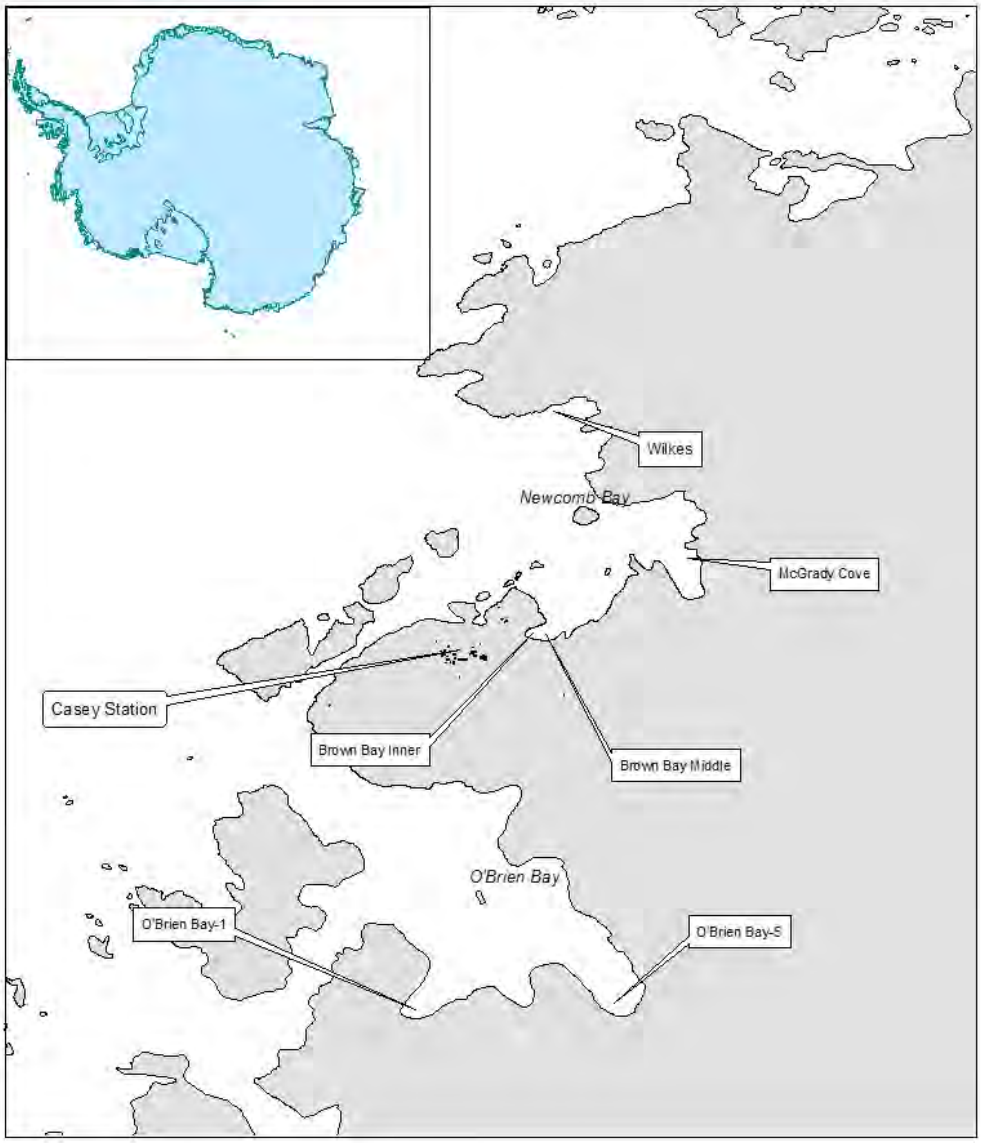

Figure 3.1: Map shows the study location in Casey, Antarctica.

3.2.2 Heavy Metal Analysis Methods

All plastic and glassware was soaked in 10% HNO_3 for 24 hours and rinsed with Milli-Q water (resistance >18 MΩ) before use.

A partial metal extraction was used as detailed in Snape (2004) and Larner (2006). Briefly, sediment was first sieved through a 2 mm plastic mesh and the component < 2 mm was used in extractions. Four hour extractions were conducted using 1.0 M HCl (prepared from analytical grade HCl 32% w/w; Univar). All extractions used 1 g of sediment to 20 ml 1 M HCl solution (1:20 w/v) and were extracted in Teflon containers on an orbital shaker for a period of 4 h. Supernatants were immediately filtered through 0.45 lm cellulose nitrate membranes (Sartorius) using a Millipore filter unit into a 70 mL HDPE container and stored at 4°C until elemental analysis using magnetic sector ICP-MS (Finnigan Element 1, Bremen, Germany, offering high spectral resolution capability) at the Central Science Laboratory, University of Tasmania, using a previously reported method Townsend (2000, 1999). A total of 20 elements were measured (Ag, Al, As, Ba, Bi, Ca, Cd, Co, Cr, Cs, Mn, Mo, Pb, Rb, Sn, Sr, Th, Tl, U, Zn). Th is a rare earth element. Triplicate analysis of individual sediment samples (range 1-14% relative standard deviation (RSD), average 5% RSD, n=10) and analysis of certified reference materials MESS-3 and PACS-2 ensured quality control. Detection limits (DL) were 3 σ of the blanks with As, Mo, Ni, Sb, and Sn sometimes below DL values of 0.4, 0.03, 0.3, 0.001 and 0.01 mg kg^{-1} dw (dw denotes dry weight) respectively. When measured concentrations were below DL, values of half the DL were used in statistical analyses.

3.2.3 TOC – Total organic carbon analysis

Approximately 2-3 g of wet sediment, was accurately weighed, dried for 24 hours at 105 °C in a crucible and re-weighed to determine water content (WC), then placed in a muffle furnace at 550 °C for 4 hours for organic content determination by Loss On Ignition (LOI, Heiri et al. (2001)). Triplicate analysis of individual samples indicated RSDs of 0.6% for WC and 2.2% for LOI (n=6).

3.2.4 Grain size

A 0.25-1 g sample of dry sediment was weighed into a clean beaker. 30 mL of 5% Sodium Hexametaphosphate (filtered to < 0.45 micron) was added and the mixture sonicated for 30 seconds. After a period of 24 hours, the sample was analysed using a Malvern Mastersizer 2000 with Hydro 2000G. For this analysis, four replicates (*n* = 4) of grain size samples were analysed. There were 17 parameters measured. These were TOC, 0.01 – 2µ, 2 - 7.8µ, 7.8 - 15.6µ, 15.6 – 31µ, 31 - 62.5µ, 62.5 – 125µ, 125 – 250µ, 250 – 500µ, 500 – 1000µ 1000 – 2000µ, Maximum phi, Minimum phi, Mean um, Sorting quartile deviation, Sorting coefficient, graphic Skewness, Inclusive graphic Skewness, Kurtosis, <2mm % and >2mm %

3.2.5 Statistical methods

The relationship between community structure and environmental variables were examined using the PRIMER program (Plymouth Marine Laboraties, UK) and included use of several methods: the BEST routine, LINKTREE and RELATE. The degree of correlation among similarity matrices was tested using RELATE analysis, a non-parametric form of the mantel test. To estimate the threshold of environmental variables for separation of groups of samples, linkage tree (LINKTREE) analysis, a non-parametric modification of multivariate regression trees by De'ath (2002), was performed using the subset of environmental variables identified by BEST analysis (Clarke and Gorley, 2006, Clarke et al., 2008). A LINKTREE analysis was applied to determine which abiotic variables represented the greatest differences among groups of samples (SIMPROF test). The other statistic reported for LINKTREE analyses is B%, which is an absolute measure of group differences from the original resemblance matrix. When B% is large, it indicates that the split group is that percentage different than the remainder of sites. An analysis of correlations among environmental variables was done and where correlations were greater than 0.95, one of the variables was removed to prevent over-parameterization.

3.3 Results

3.3.1 Relationships between biological and environmental parameters

Multivariate relationships between biological and environmental parameters were examined using a variety of methods. The MDS of the meiofaunal communities (abundances averaged over plots) can be seen in Figure 3.2. The relationship between samples based on all environmental variables measured is shown in a PCA analysis, which showed clear differences between locations (Figure 3.3a), but also that when all environmental variables that were measured are considered, the resulting pattern is very unlike the biological pattern of meiofaunal communities. Based on all measured environmental variables, O'Brien Bay-1 was very different to all other locations. O'Brien Bay-5 and McGrady Cove were very similar to each other as were Brown Bay Inner, Brown Bay Middle and Wilkes. Wilkes showed the biggest difference within locations (Figure 3.3a). An MDS of all environmental variables (based on a Euclidean distance similarity measure) showed very similar patterns to the PCA of environmental variables (Figure 3.3b).

Several methods were used to try to find a subset of the environmental variables that better 'explained" or matched the meiofaunal patterns.

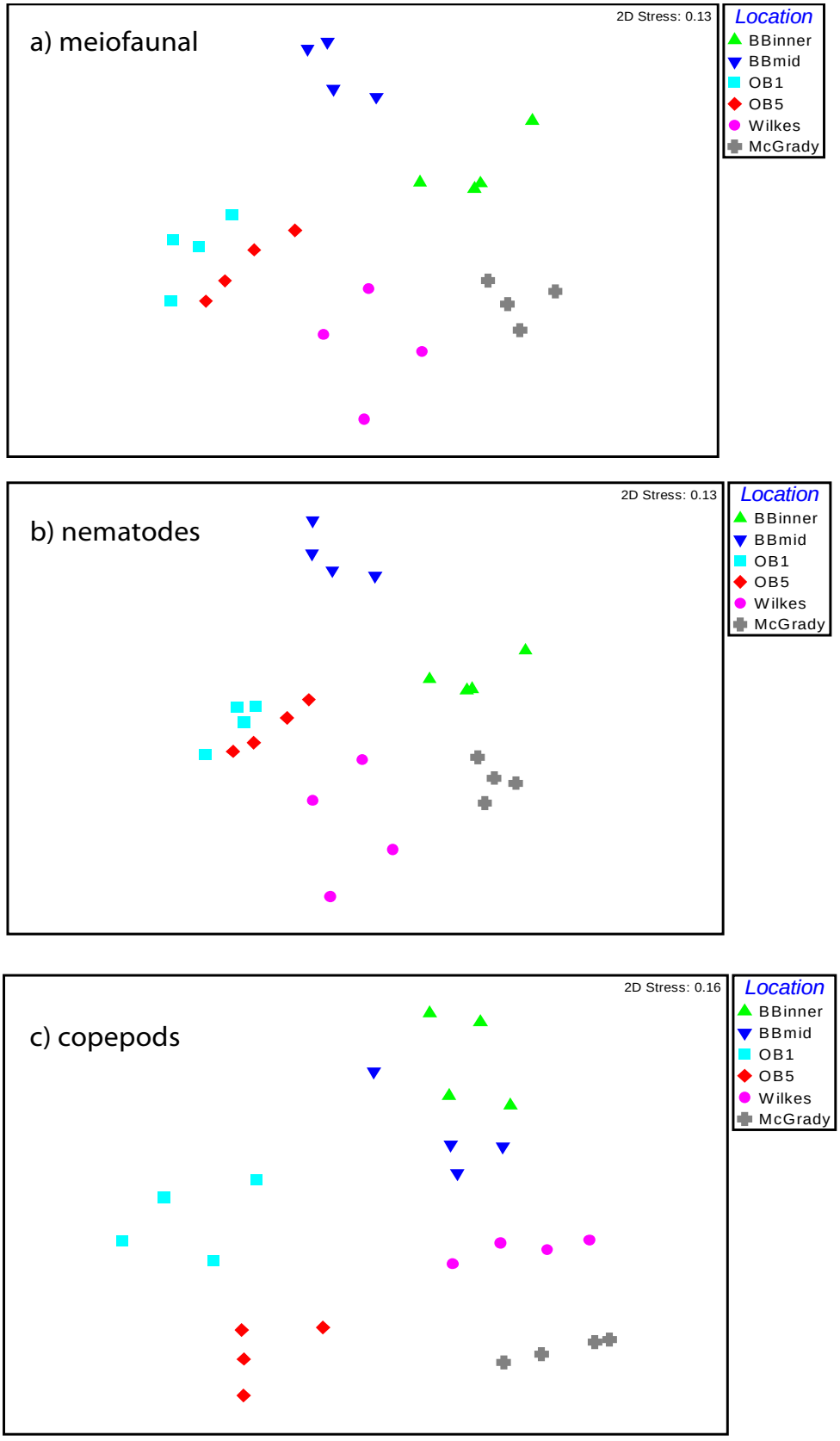

Figure 3.2: nMDS ordinations of biota (a) meiofaunal, b) nematodes only and copepods only.

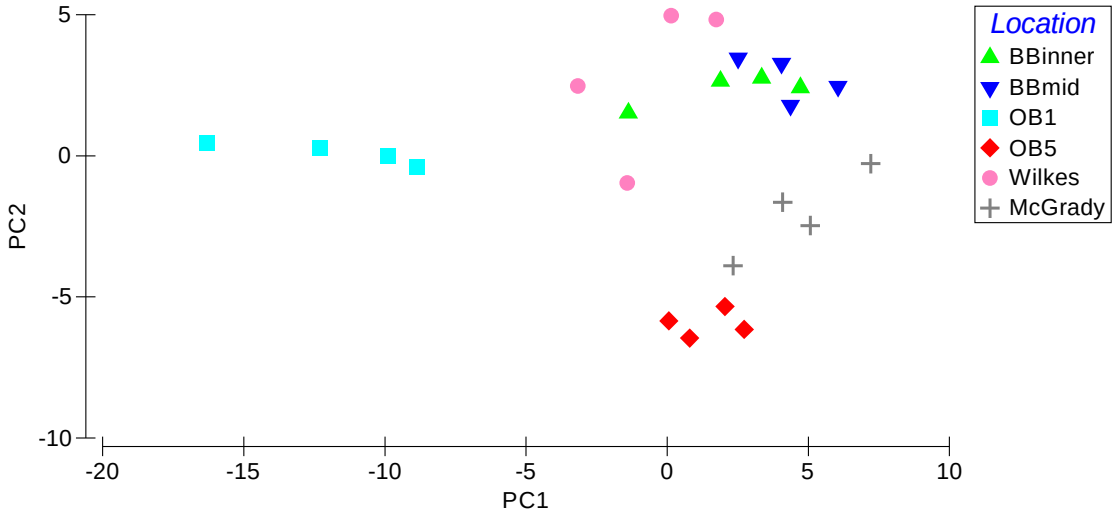

Figure 3.3a: PCA ordination of all environmental variables between locations (total variance explained by the first two principal components = 52.8 %).

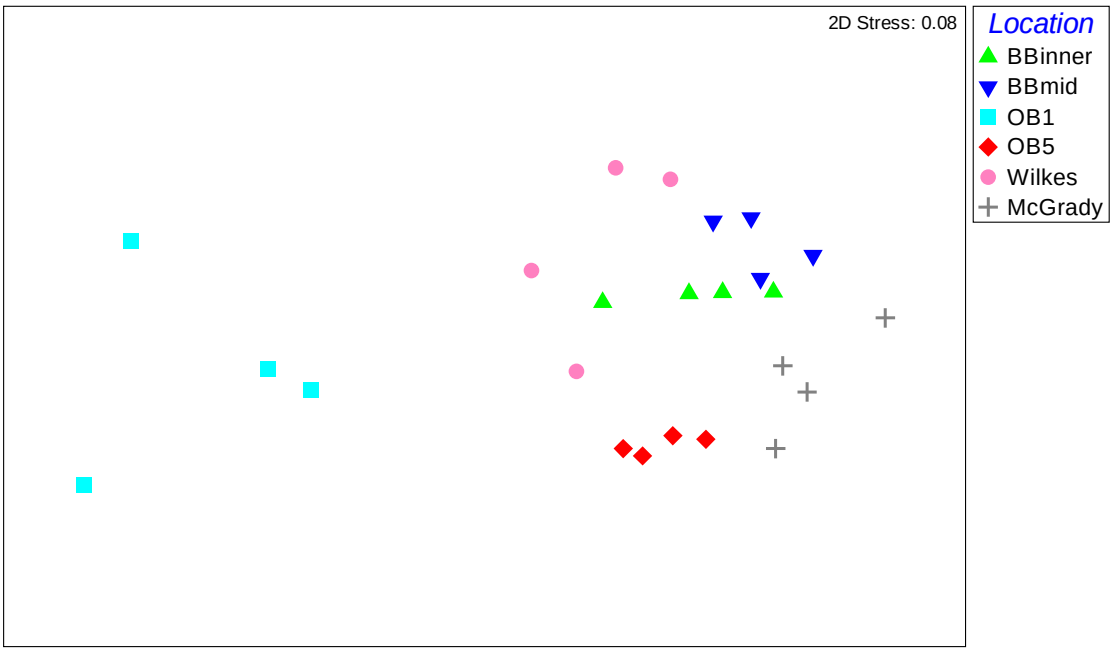

Figure 3.3b: nMDS of environmental variables

Table 3.1: Results of PCA. Eigenvectors for all variables, eigenvalues and percentage of variation are given.

Variable	**PC1**	**PC2**
Rb	-0.142	0.103
Sr	-0.146	-0.097
Mo	-0.121	-0.137
Ag	-0.089	-0.084
Cd	-0.092	-0.171
Sn	-0.059	-0.151
Ba	-0.144	-0.056
Tl	-0.079	-0.212
Pb	-0.083	-0.153
Bi	-0.064	0.003
Th	-0.128	0.073
U	-0.128	-0.133
Al	-0.102	0.126
Cr	-0.129	0.054
Co	-0.108	0.185
Zn	-0.130	-0.130
As	-0.120	-0.101
TOC (LOI)	-0.127	-0.154
0.01 - 2	-0.103	0.188
2 - 7.8	-0.121	0.166
7.8 - 15.6	-0.143	0.106
15.6 - 31	-0.154	-0.019
31 - 62.5	-0.136	-0.128
62.5 - 125	0.066	-0.098
125 - 250	0.156	-0.007
250 - 500	0.151	0.022
500 - 1000	0.115	0.052
1000 - 2000	0.117	-0.005
Maximum phi	-0.135	0.133
Minimum phi	-0.130	-0.083
Mean um	0.160	0.002
Sorting quartile deviation	-0.037	0.152
Sorting coefficient	0.043	-0.145
graphic Skewness	-0.129	0.055
Inclusive graphic Skewness	-0.136	0.057
Kurtosis	0.067	-0.070
<2mm %	-0.065	-0.024
>2mm %	0.065	0.024
Eigenvalues	36.4	12.0
%Variation	52.8	17.4

The relationships between biological distributions and single environmental variables were examined by superimposing environmental variables onto the MDS of the meiofaunal assemblages (Figure 3.4 and 3.6). Many metals were highly inter-correlated, particularly Pb with Fe, Cu, Ni, Sb, Sn, Sr, Mn and Zn. Some of these highly correlated metals were excluded from further analyses to prevent over parameterization, including Cu, Fe, Cr and Sn.

There were clear relationships between many of the metals and the meiofaunal assemblages. Some important taxa (*Paralinhomoes*, *Halalaimus*, *Chromadorina*) had positive correlations with selected metals. In Brown Bay Inner, the strongest correlation was seen between *Paralinhomoes* and Sn ($r^2 = 0.626$, $p = 0.097$). The Genus *Halalaimus* had a strong correlation with Cr at Wilkes ($r^2 = 0.718$, $p = 0.045$) and with Zn at O'Brien Bay-5 ($r^2 = 0.945$, $p < 0.001$). While the genus *Chromadorina* was found to have a strong correlation with Zn at Brown Bay Middle ($r^2 = 0.569$, $p = 0.141$) and McGrady Cove ($r^2 = 0.721$, $p = 0.043$). The Zosimidae were seen as sensitive taxa. They were founds to have a negative correlation with Al ($r^2 = -0.0585$, $p = 0.128$), Cd ($r^2 = -0.730$, $p = 0.040$) and Zn ($r^2 = --0.700$, $p = 0.053$) in Brown Bay Middle.

The potentially impacted locations, excluding Wilkes, all had higher concentrations of heavy metals (in particular Pb, Ag, As, Cu, Fe, and Sn) than the control locations (Figure 3.5). Wilkes had relatively high levels of Cd and Zn. The heavy metals tended to have their greatest concentrations at locations on the top right side of the ordination, which are represented by Brown Bay Inner, Brown Bay Middle (Figure

3.4) although McGrady Cove also had relatively high levels of some metals such as Ag, U, Cd, and Zn. Vector plots of environmental variables overlaid on the MDS of meiofaunal communities show the strong relationship between many metals and meiofaunal communities. For example there were strong positive correlations between the Brown Bay samples and many of the anthropogenically derived metals such as Pb, Sn, Zn, Fe, Cu, Sn. By comparison, meiofauna from McGrady Cove showed strong correlations with metals that most likely to have been derived from local mineralogy such as Ba, Th, Cr, Co. (Figure 3.8a and Figure 3.8b). All sites had similar proportions of fine sediment, < 63 µm, but Brown Bay had less fine and coarse sands than O'Brien Bay (Figure 3.6). The average grain size was smaller at Brown Bay and McGrady Cove, which were situated in Newcomb Bay, while the coarser and sandiest sediments were mostly found in O'Brien Bay. Variations in grain size contributed less to the meiofaunal distributional patterns than the heavy metals (Figure 3.7). Many grain size classes were highly variable within locations (Figure 3.7).

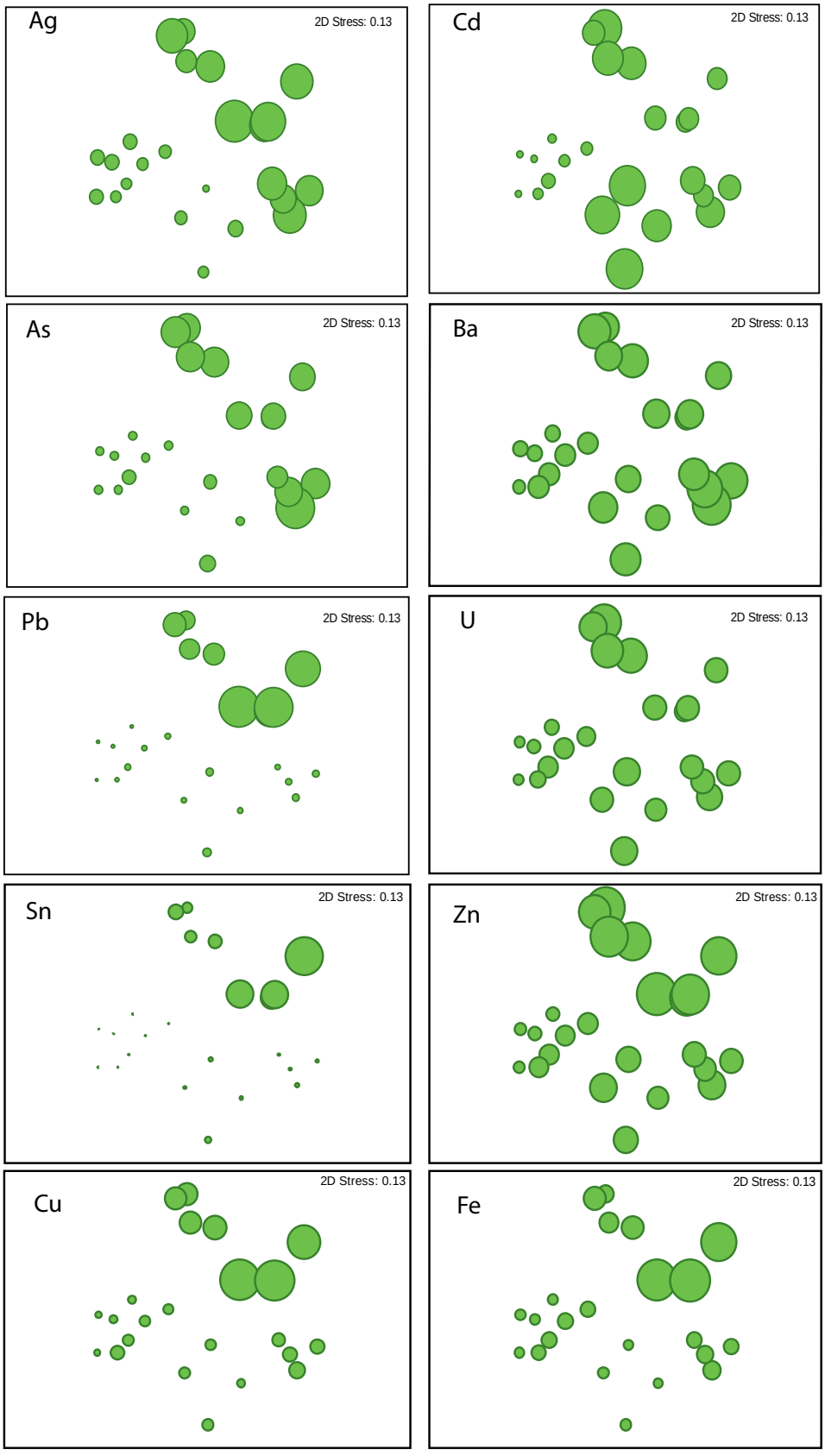

Figure 3.4: nMDS ordinations of average value of heavy metals superimposed onto the MDS of meiofauna assemblages.

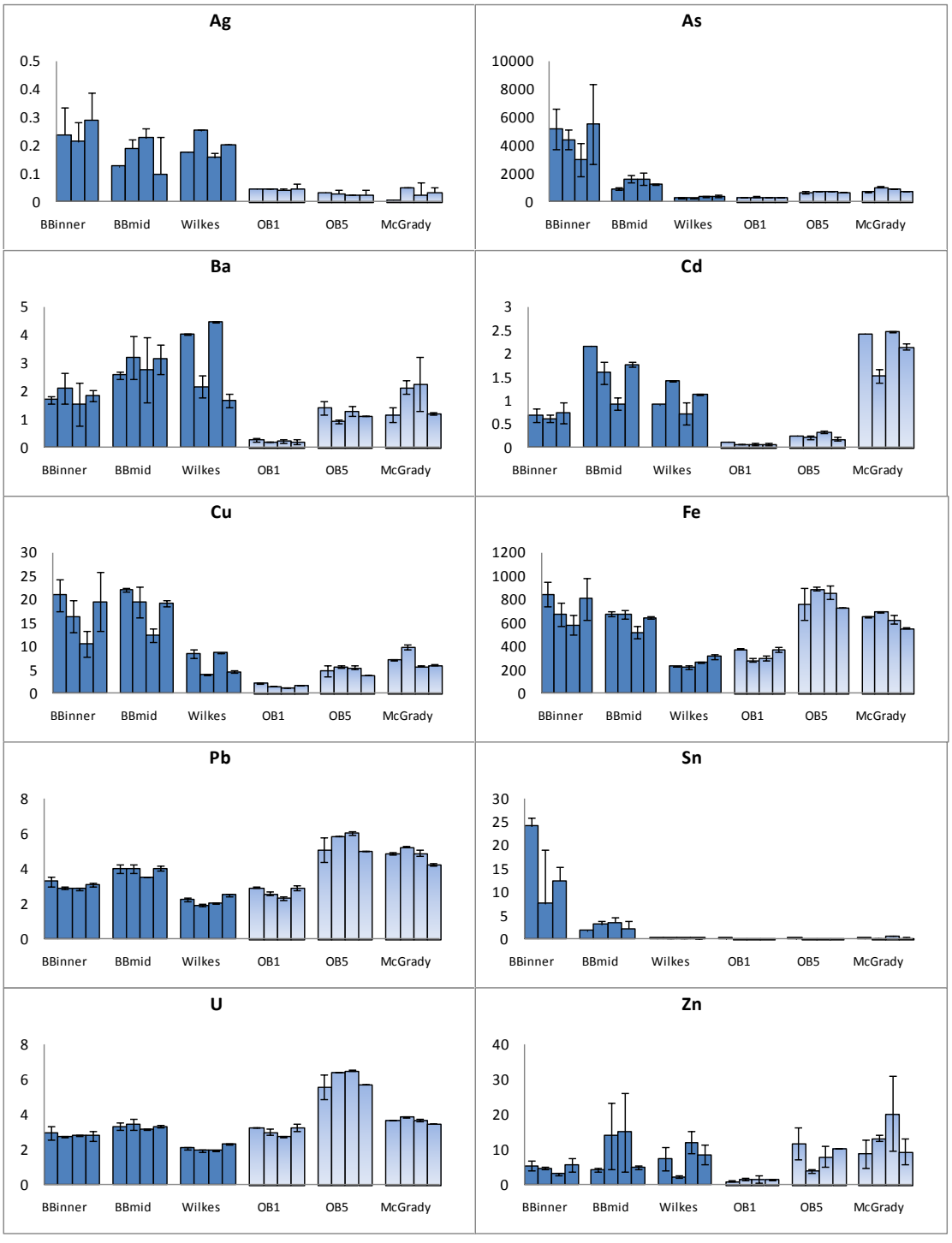

Figure 3.5: Histograms of mean values of selected heavy metals in each of four plots (individual bars) at each location. Light shaded bars represent control locations; dark shaded bars represent potentially impacted locations.

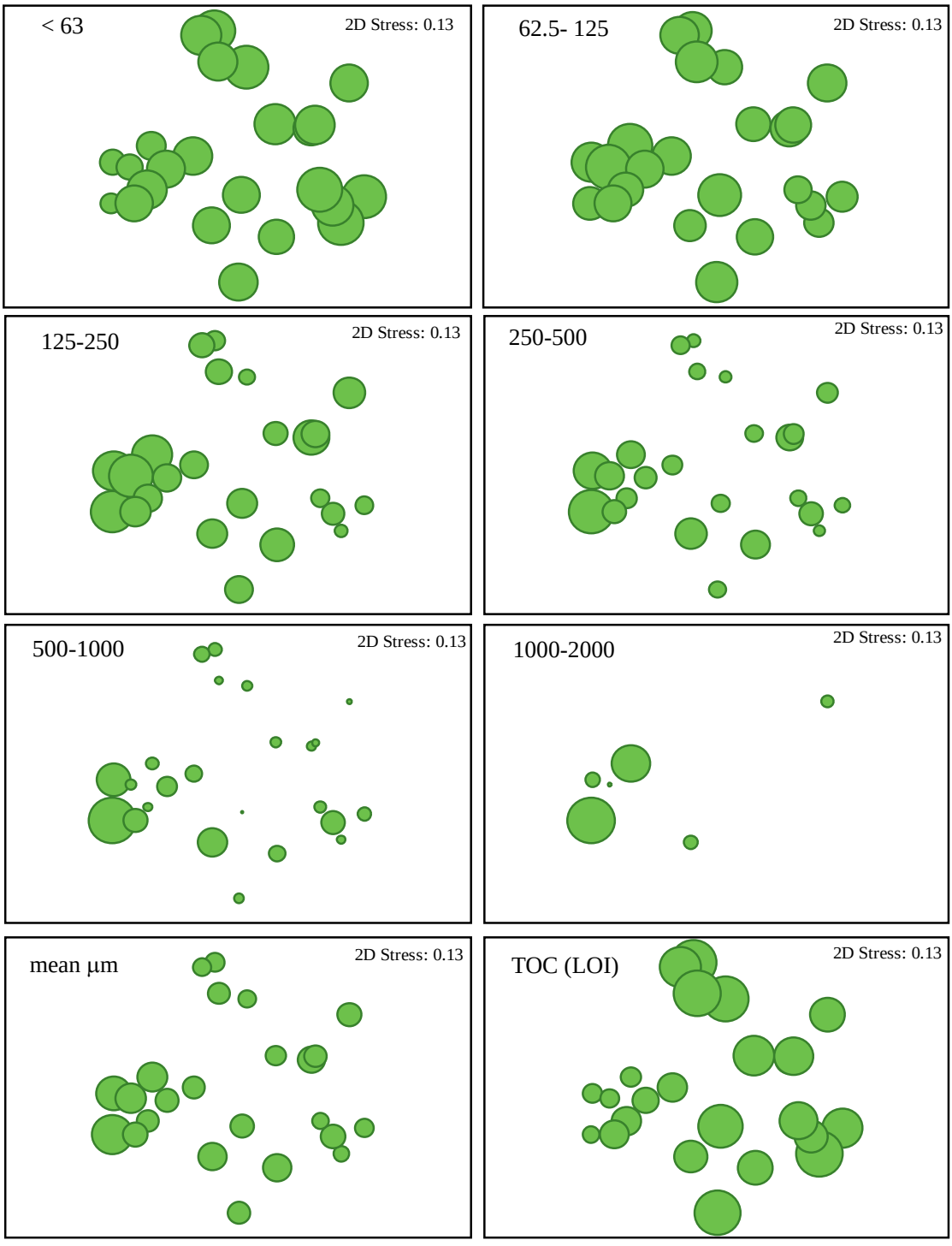

Figure 3.6: nMDS ordinations of average value of sediment properties; grain size (µm) and TOC (LOI) superimposed onto the nMDS of meiofauna assemblages.

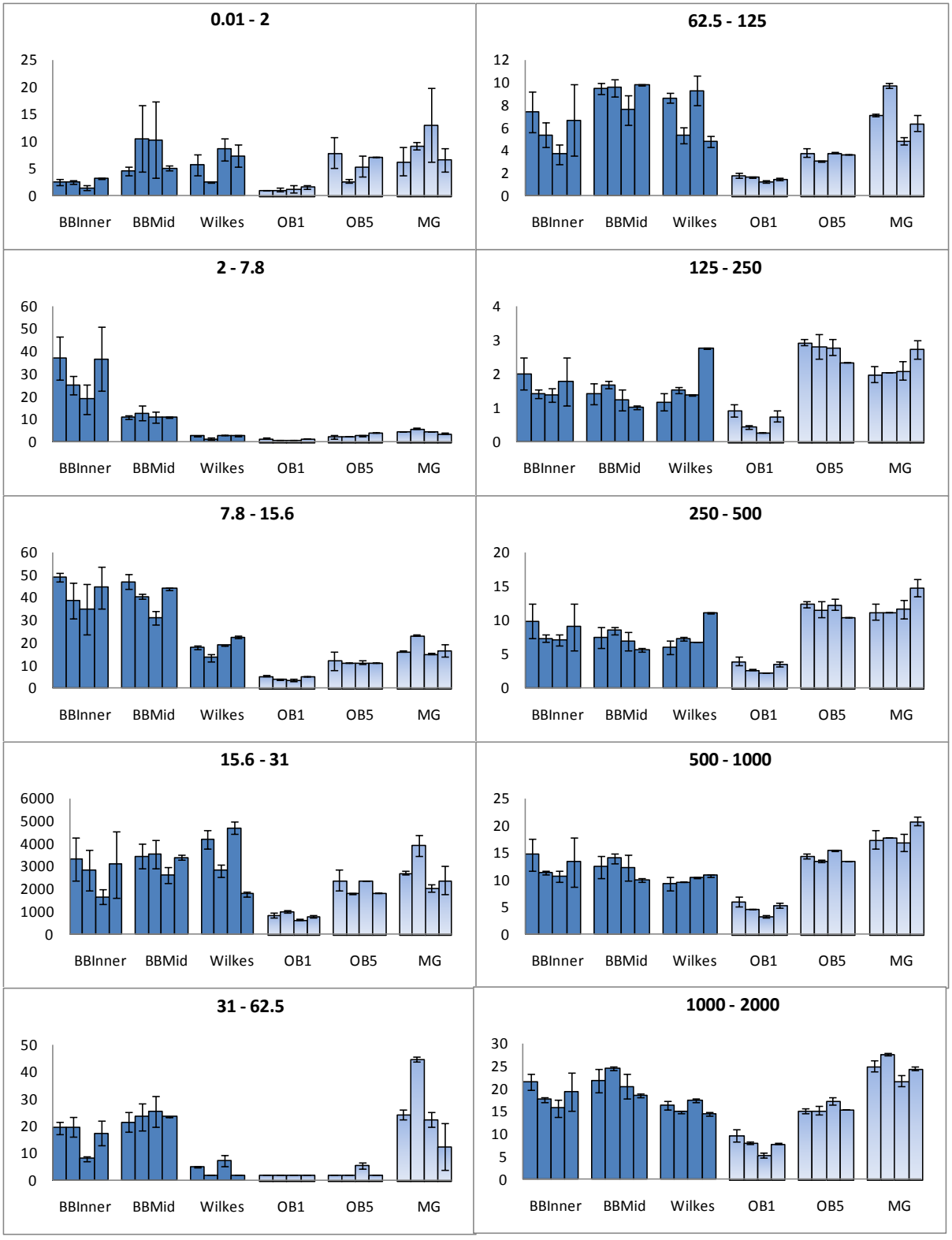

Figure 3.7: Histograms of mean values of sediment grain size variables in each of four plots (individual bars) at each location. Light shaded bars represent control locations; dark shaded bars represent potentially impacted locations.

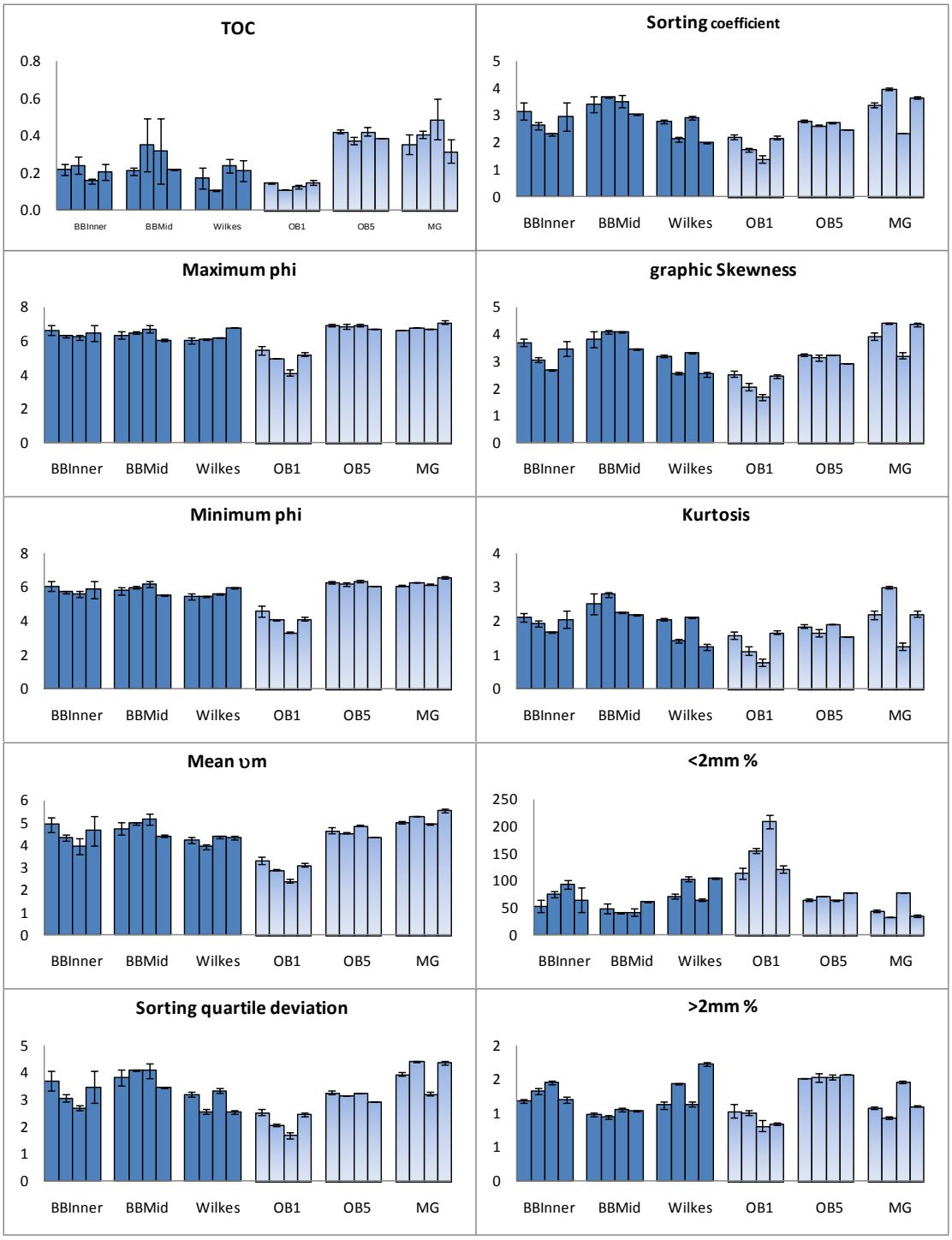

Figure 3.7(cont.): Histograms of TOC and mean values of sediment grain size variables in each of four plots (individual bars) at each location. Light shaded bars represent control locations; dark shaded bars represent potentially impacted locations.

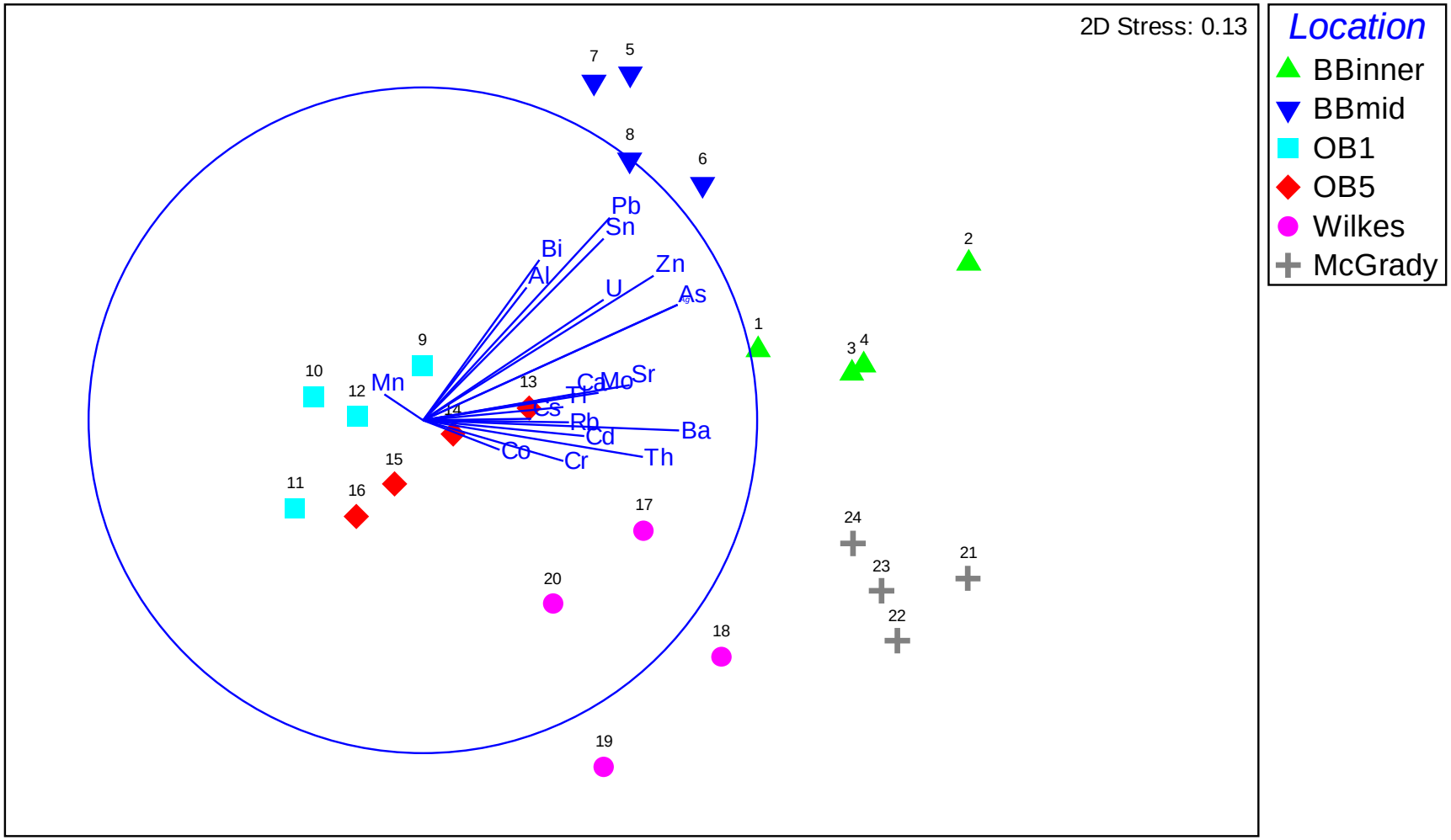

Figure 3.8a: Combination of nMDS and vector shows locations were influenced by heavy metals.

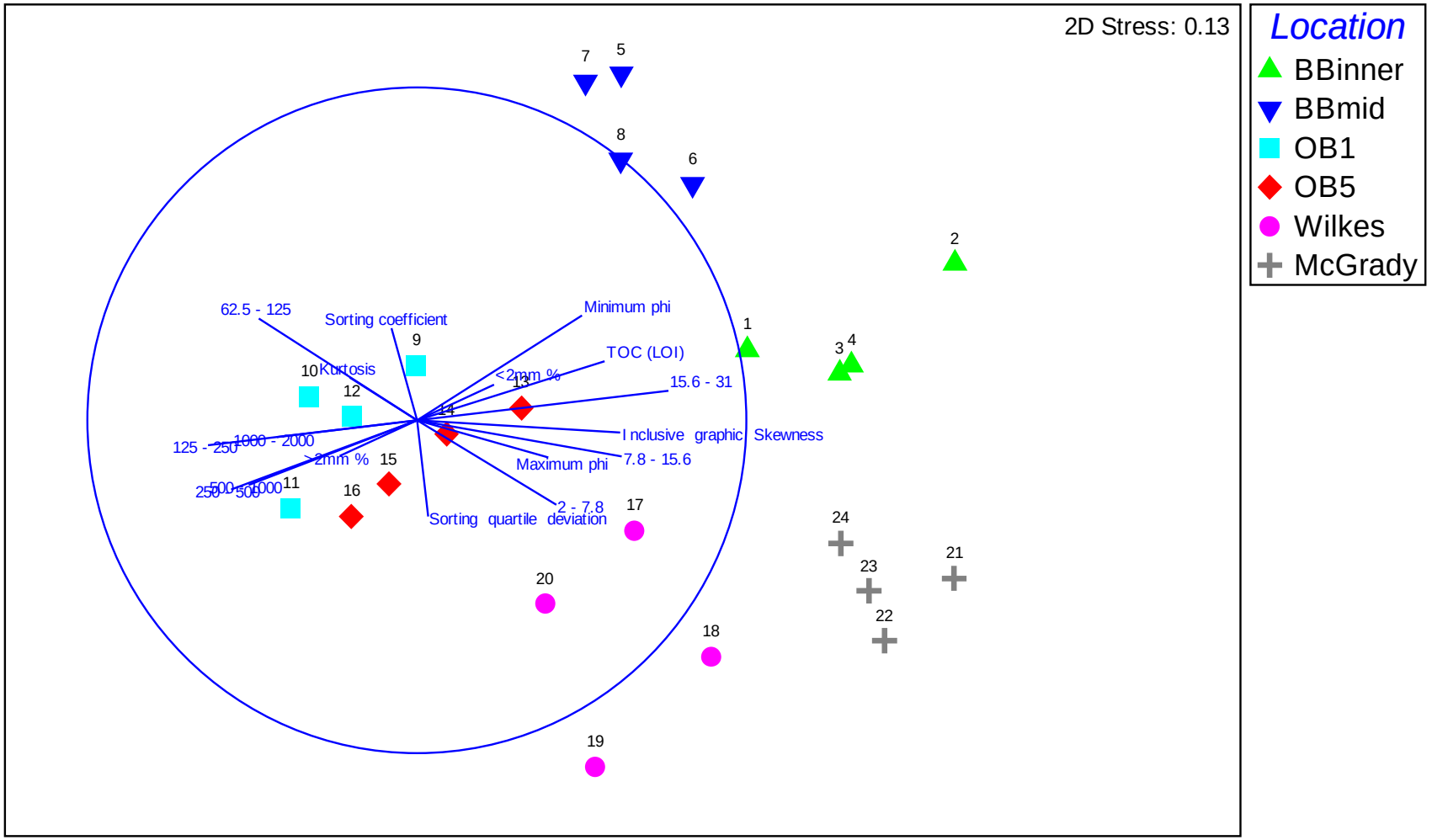

Figure 3.8b: Combination of nMDS and vector shows locations were influenced by grain size and other sediment variables.

The BEST analysis using BVSTEP (Clarke and Gorley, 2006) was used to explore relationships between groups of environmental variables and the biotic similarity matrix, to find a combination of variables that most closely matched the biological patterns. BVSTEP consistently defined a group of 37 variables including minerals and metals that contributed to combinations of variables with good correlations. The best combination from BVSTEP was of five metals (Table 3.3). The MDS of these five metals shows a pattern with many similarities to the biotic MDS (Figure 3.9a), however, the locations McGrady Cove and Brown Bay Middle are overlapping. By including three additional variables (Cd, Pb and Zn) the resulting MDS now clearly separates McGrady Cove and Brown Bay Inner without overly affecting the remaining pattern between locations (Figure 3.9b). The RELATE analyses (testing matched resemblance matrices) showed that the combination of Cd, Pb and Zn in the group of Ag, Sn, Ba, U, and As had a very similar correlation to the optimal combination selected using BVSTEP ($\rho = 0.559$) (Table 3.2).

Table 3.2: BVSTEP results shows a selection of environmental variables best explaining meiofauna community pattern.

No. Variables	**rho**	**Selections**
5	0.575, $p < 0.001$ (BVSTEP)	Ag, Sn, Ba, U, and As
8	0.559, $p < 0.001$ (RELATE)	Ag, Sn, Ba, U, As, Cd, Pb, and Zn

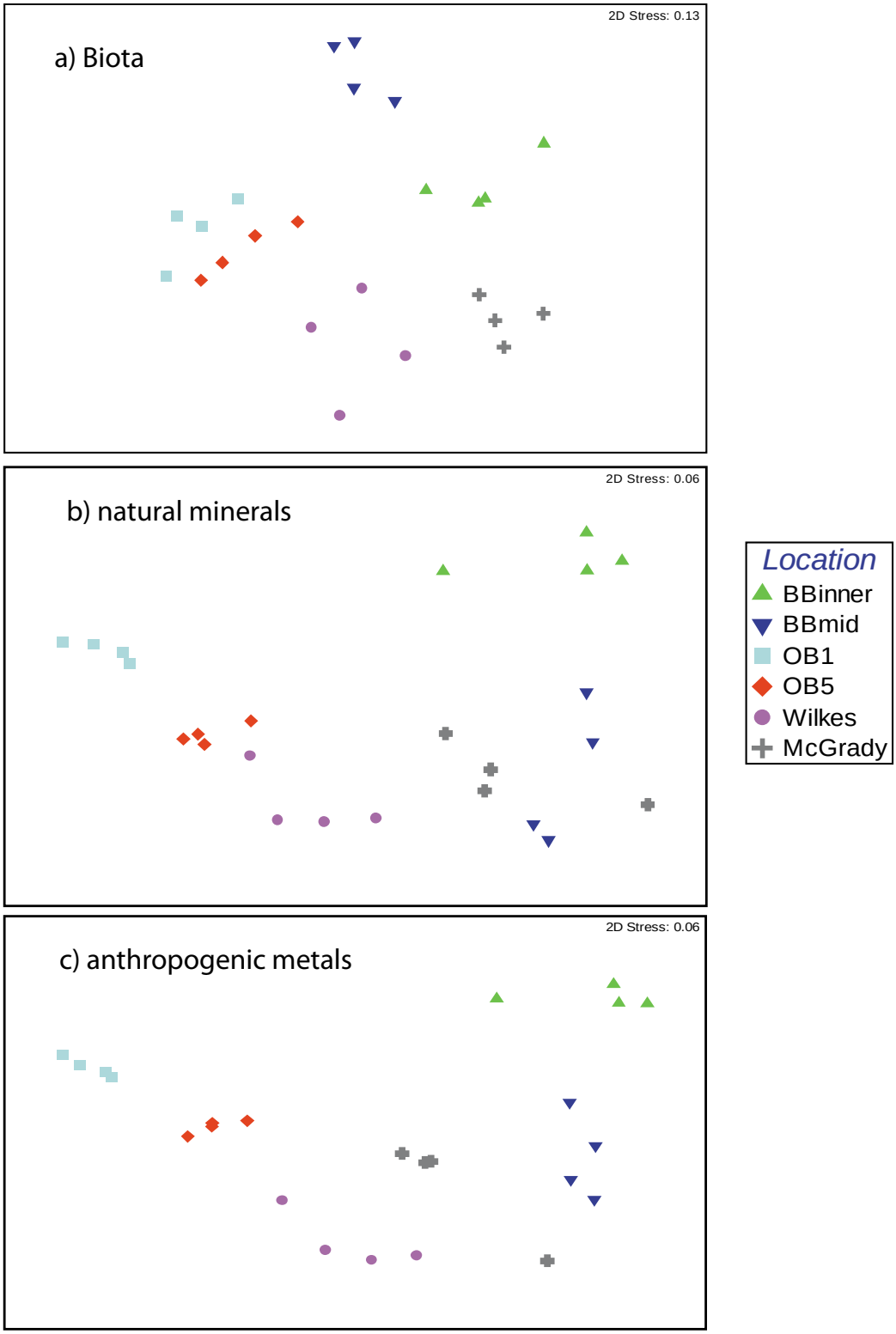

Figure 3.9: nMDS ordinations of a) biota b) selected natural minerals (Ag, Sn, Ba, U and As) and c) anthropogenic metals (Cd and Zn).

The LINKTREE analysis was used to find the most effective way of describing the biological-environment relationships relative to the successive use of single variables. In the first LINKTREE analysis, the concentrations of Ag resulted in separation of locations into two groups; however, one of the Wilkes samples was split between groups and included with Brown Bay and McGrady Cove (Figure 3.10a). The concentration of Ag at Wilkes, however, was much lower than at Brown Bay and McGrady Cove, and generally the difference in Ag concentrations between the two groups is not large. A second LINKTREE analysis excluding Ag showed that As produced a very similar split into two groups but here the Wilkes samples now grouped together (Figure 3.10b). The McGrady Cove (control) samples were grouped with Brown Bay Inner, based on the concentration of U (Figure 3.10b). As this is likely to have had little effect on the communities due to the very low concentration and only relatively small differences between locations, a third LINKTREE analysis was undertaken excluding Ag and U (Figure 3.10c). The results of this LINKTREE showed that all sites were well separated into four main groups (SIMPROF test, $P < 0.01$; Figure 3.10c). Here, the first split separate sites Wilkes, O'Brien-1 and O'Brien-5 from sites McGrady Cove, Brown Bay Middle and Brown Bay Inner at B% = 86.8, with the As concentration less than 7.32 ppm for the former site group and greater than 8.14 ppm for the latter (Figure 3.10c, Table 3.3). The next division separates samples from Wilkes from those in O'Brien Bay at B% = 61.4, with a higher Cd concentration in the former sites (Figure 3.10c, Table 3.4). The control location (McGrady Cove) was separated from Brown Bay locations at B% = 79.1, with the former sites showing lower concentrations of Pb than the latter, but a big difference between the two groups (Figure 3.10c, Table 3.3). The split at G, B% = 64.2 divides Brown Bay Middle and Brown Bay Inner. Pb and Sn

concentrations level were higher at Brown Bay Inner and there were also some grain size differences (Table 3.3, Figure 3.10c). The nMDS of meiofaunal assemblages was then split based on LINKTREE analyses showing how the locations were separated (Figure 3.11).

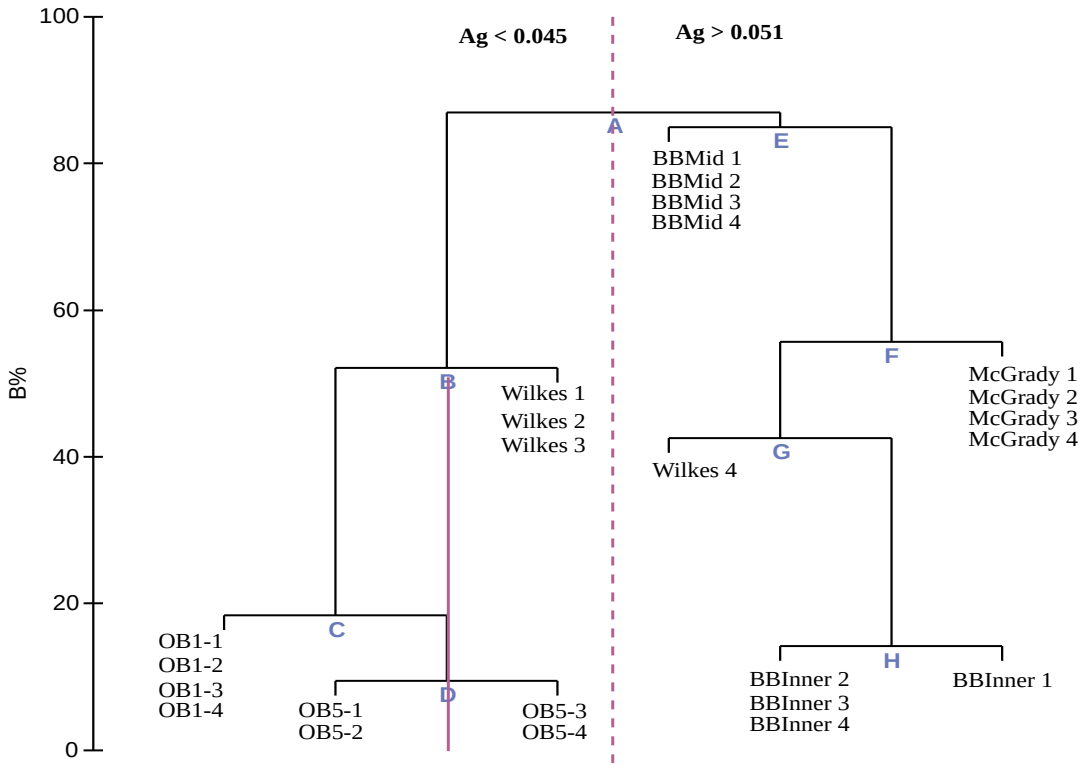

Figure 3.10a: LINKTREE analysis showing divisive clustering of sites from all environmental variables based on silver (Ag).

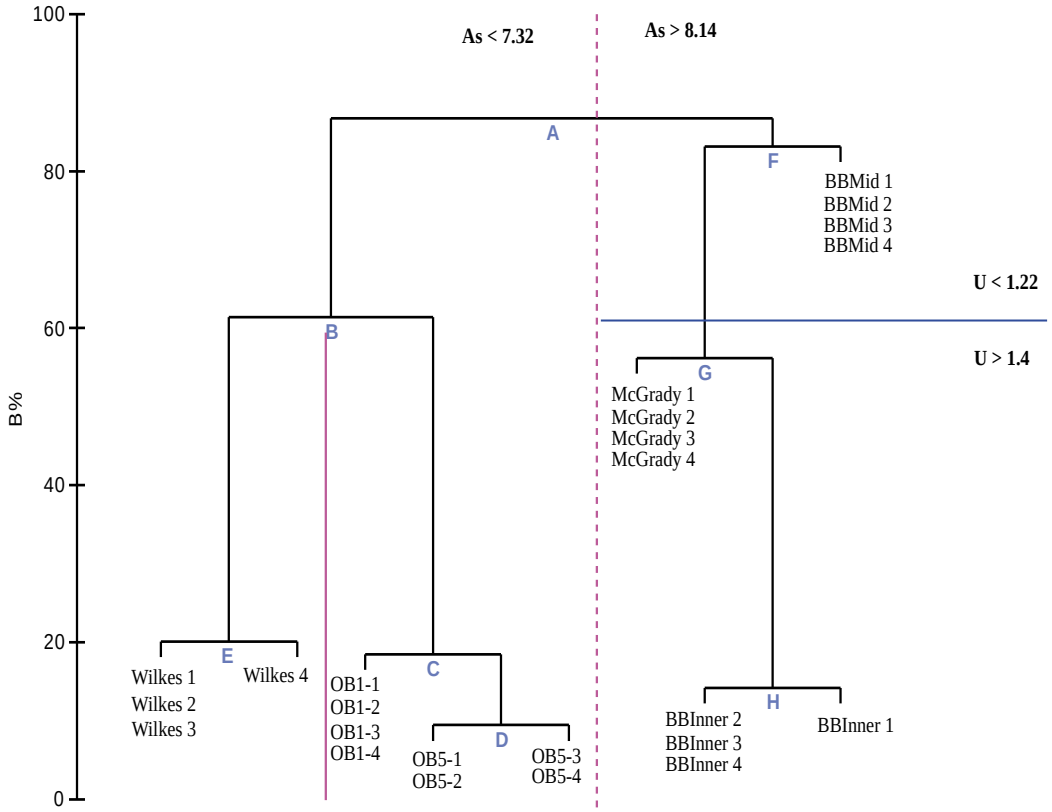

Figure 3.10b: LINKTREE analysis showing divisive clustering of sites from all environmental variables based on Arsenic (As).

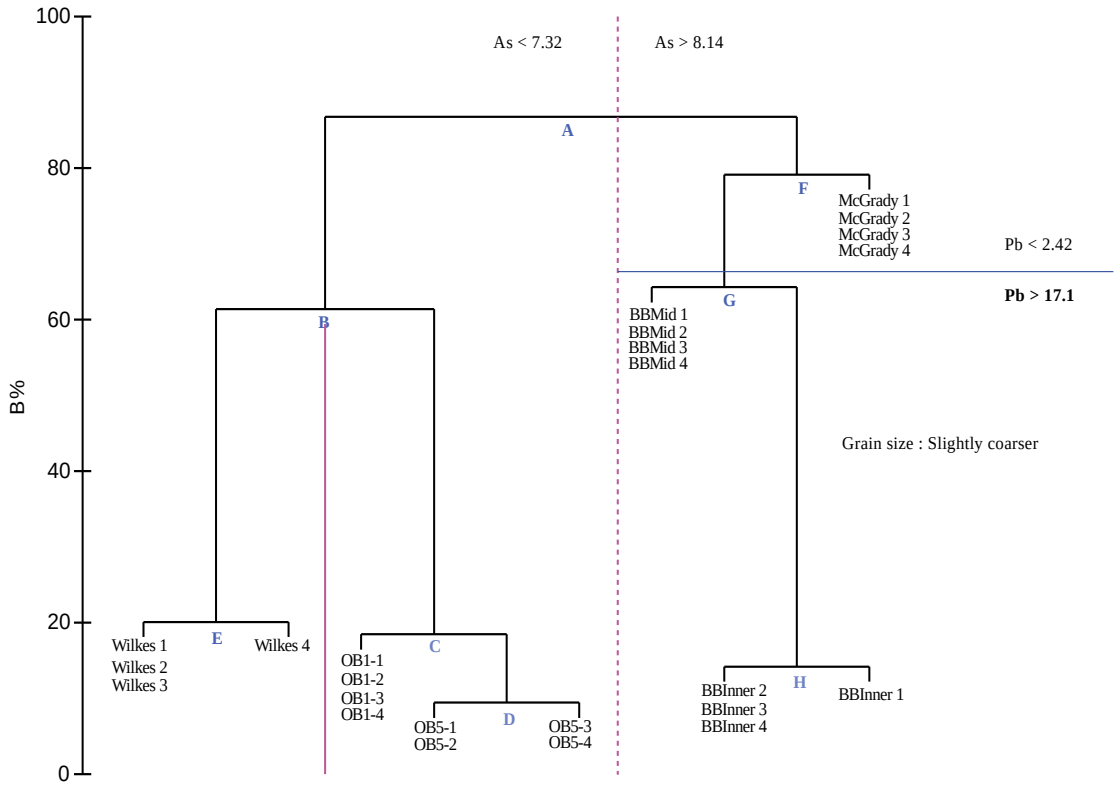

Figure 3.10c: LINKTREE analysis showing divisive clustering of sites from all environmental variables with the exclusion of rare earth and some metals (Ag and U) based on As.

Table 3.3: LINKTREE and SIMPROF test result.

Split	Variables	Location
A $\pi = 4$ P= 0.1% R= 0.59 B% = 86.8	B: As < 7.32 F: As > 8.14	Wilkes, OB 1, OB 5 McGrady Cove, BB Mid, BB Inner
B $\pi = 5.35$ P= 0.1% R= 1.0 B% = 61.4	C: Cd < 0.334 TOC <3.83 Mn > 8.93 Zn < 12.1 Sn < 0.0249 E : Cd > 1.53 TOC (LOI) >4.85 Mn < 7.84 Zn > 13.5 Sn > 0.144	OB 1, OB 5 Wilkes
C $\pi = 1.13$ P= 0.1% R= 0.81 B% = 18.4	C: Pb < 0.576 Sn < 0.01 Zn < 5.35 Cd < 0.127 Cr < 1.73 Mn < 10.3 Maximum phi < 6.98 2 -7.8 < 4.02 Sorting quartile deviation < 1.04 Sorting coefficient > 0.489 7.8 - 15.6 < 6.08 Inclusive graphic Skewness < -0.306 125 - 250 > 28.9 Kurtosis > 1.08 15.6 - 31 < 9.82 250 - 500 > 10.3 TOC (LOI) < 1.78 1000 - 2000 > 0.00592 D: Pb > 0.983 Sn > 0.0192 Zn > 11.2 Cd > 0.189 Cr > 4.07 Mn > 14.7 Maximum phi > 8.14 2 - 7.8 > 10.4 Sorting quartile deviation > 1.52 Sorting coefficient < 0.349 7.8 - 15.6 >13.5 Inclusive graphic Skewness > -0.12 125 - 250 < 16.8	OB 1 OB 5

	Kurtosis < 0.893 15.6 - 31 > 15.2 250 - 500 < 7.37 TOC (LOI) > 3.08 1000 - 2000 < 0	
F π = 5.62 P= 0.1% R= 0.66 B% = 79.1	G Pb > 17.1 Zn > 31.2 Mn < 11.6 Sn > 1.9 62.5 - 125 > 16.1 7.8 - 15.6 < 14.7 2 - 7.8 < 9.89	McGrady Cove, Brown Bay Inner, Brown Bay Middle
	[21-24] Pb < 2.42 Zn < 23.4 Mn > 12 Sn < 0.272 62.5 - 125 < 12.6 7.8 - 15.6 > 16.9 2 - 7.8 > 11.2	McGrady Cove 1, McGrady Cove 2, McGrady Cove 3, McGrady Cove 4
G π = 6.2 P= 0.1% R= 1 B% = 64.2	[5-8] Pb < 27.5 Sn < 3.64 Mn > 10.1 As > 21.4 Cd > 0.937 Sorting coefficient > 0.481 Sorting quartile deviation < 1.06 Minimum phi > 2.18 TOC (LOI) > 7.6 Kurtosis > 1.02	BB Middle
	H Pb > 51.1 Sn > 7.6 Mn < 9.37 As < 19.8 Cd < 0.829 Sorting coefficient < 0.442 Sorting quartile deviation > 1.18 Minimum phi < 2.12 TOC (LOI) < 7.4 Kurtosis < 1.01	BB Inner

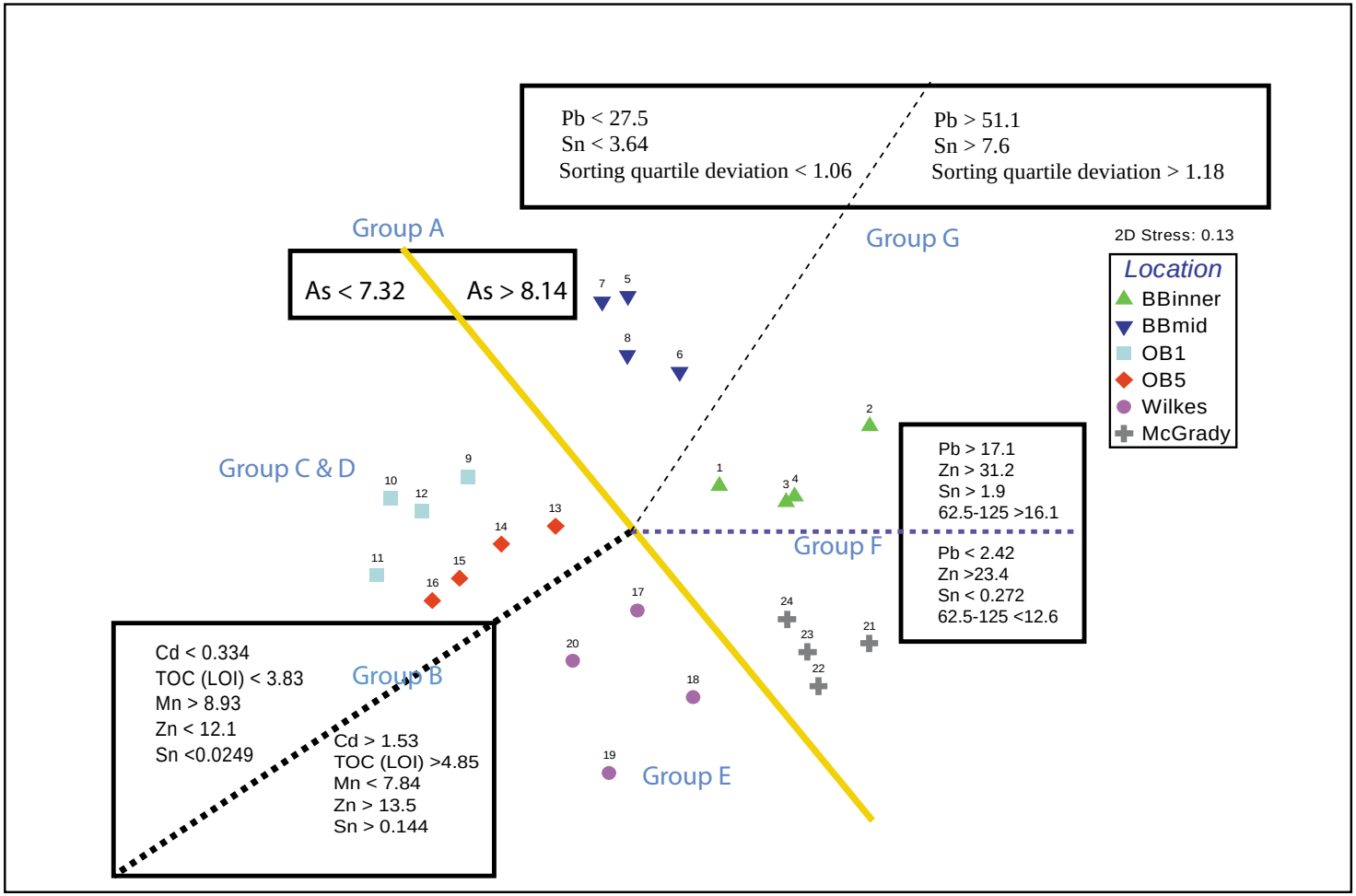

Figure 3.11: Splitting group of nMDS based on LINKTREE analyses.

3.4 Discussion

The aim of this study was to assess the effects of sediment properties such as heavy metals and grain size on the structure of the meiofaunal community assemblages at six different locations that were categorized as either controls or anthropogenically disturbed. By using multivariate techniques, differences in meiofaunal assemblages between control and disturbed locations at Casey Station were found to be closely correlated with concentrations of metals in sediments. Similar correlations between soft-sediment assemblages and heavy metal contaminations were found in a study at Casey Station by Stark (2003b), where the correlation between the biota matrix and a group of five metals (Cd, Cu, Pb, Sn and Zn) was 0.65. Surprisingly, the meiofaunal community patterns here showed little correlation with sediment grain size and TOC content. Grain size and TOC did not seem to be a major influence on the meiofaunal assemblages and there were no clear relationships with sediment grain size or TOC in spite of grain size having previously been shown to be very influential on meiofaunal community structure (Gyedu-Ababio et al., 1999, Somerfield et al., 1994, Soetaert et al., 1995).

Sediment grain size and TOC are known to influence the concentration of metals in sediments (Lakhan et al., 2003, Stark et al., 2003b). Although McGrady Cove was a control location, it had similar TOC and grain size characteristics to the disturbed locations in Newcomb Bay. In contrast, O'Brien Bay had lower TOC concentrations and a coarser grain size. This may reflect differences in mineralogy and sedimentation patterns between Newcomb Bay and O'Brien Bay. The disturbed locations, however,

were distinguished by fine sediments and high TOC and generally had much higher concentrations of metals. For example, all sites in Brown Bay have high proportions of very fine grain size (< 63 μm) and higher concentrations of Pb and Sn than the controls. These grain size and TOC distributions are consistent with the observations of Stark et al (2003b), who also noticed that locations with finer sediments tended to have greater concentrations of TOC.

However, Wilkes (old dump site), which is one of the disturbed locations, contained a higher proportion of coarse grain size sediment (> 500 μm) than other sites in Newcomb Bay but still had a higher TOC concentration than O'Brien Bay, which is similar to other disturbed locations (Figure 3.7). A positive relationship between coarse sediment and high TOC has been associated with low abundances of nematodes (Gyedu-Ababio et al., 1999). In the Casey area, this relationship was only observed at Wilkes while at O'Brien Bay there was less TOC and a higher abundance of nematodes (Figure 3.6). These results thus suggest that the meiofaunal community at Casey Station was not heavily influenced by sediment grain size.

In contrast to grain size and TOC, metal concentrations were found to have a strong influence on the community structure. Both anthropogenic and non-anthropogenic metals have been found to be significantly correlated with differences in soft-sediment assemblages (Somerfield et al., 1994). The multivariate analyses used here showed a distinctive relationship between heavy metal concentration and meiofaunal community structure. Ba, U, Sn, As and Ag were found in both control and disturbed locations in this study and so can be considered to be of non-anthropogenic origin and may have originated from erosion of soils (Dalto et al., 2006). Sn, Pb, Cu, Zn, Cd and

Fe are considered to be of anthropogenic origin as they were found in high concentrations at the disturbed locations only. Fuel spills, heavy metals/metalloids (typically Cu, Pb, Zn, Cd, Hg, As) and polychlorinated biphenyl (PCB) contamination are derived from abandoned waste disposal sites (Tin et al., 2008). Past studies had shown that the high concentrations of Cr, Zn, As, and Cd are clearly correlated with low diversity of meiofauna (Schratzberger et al., 2001) and may indicate anthropogenic impact at locations where these metals are present. These anthropogenic heavy metal contaminations such as Sn, Cu, Pb and Fe have also been found to have an influence on the benthic diatom community from Brown Bay (Cunningham et al., 2003b).

In the meiofaunal community, differences have been observed between nematodes and copepods in response to contaminant stress such as different types of heavy metal contamination. For example, low abundances of nematodes were observed in Portosole Harbour, Mediterranean, where concentrations of Zn, Pb, Cu, As and Cd in the sediments were high (Moreno et al., 2009). Similarly, in this study, high concentration of these heavy metal and low mean abundance of meiofauna were observed at Brown Bay, Wilkes and McGrady Cove. In this current study, the genus *Halalaimus, Sabatieria* and *Pirrickia* may be tolerant to metal pollution since these genera were commonly found in locations with high concentration of Ag, Pb, Sn, Zn and Cd. Somerfield *et al* (1994) demonstrated strong relationships between nematode community structure and metal concentrations. Their study suggested that several species, such as *Ptycholaimellus ponticus, Sabatieria pulchra*, *Molgolaimus demani* and *Axonolaimus paraspinosus* were able to tolerate a wide range of metal

concentrations. It is possible that these species had developed a tolerance towards metal pollution.

An attempt was made to identify which species were good indicators of disturbance, either due to their sensitivity, as inferred from absence at disturbed locations, or due to their tolerance to pollutions, as inferred from being abundant at disturbed locations. In addition, the important taxa of disturbed and controlled locations were also identified in Chapter 2. The species which appear to be most sensitive are: *Halalaimus* (nematodes) and *Zosimidae* (harpacticoid copepods). They were both found at O'Brien Bay. This finding supports the results by Essink and Romeyn (1994) whereby *Halalaimus* usually inhabits less stress and stable environment. However, this is not in agreement with Gyedu-Ababio et al. (1999), they noted that *Halalaimus* has high abundant at sewage output point and location with highest metal pollution at an estuary in South Africa. This may be due to high organic matter (high food availability) in the seawage area. While, the taxa which were characterised as opportunistic species in polluted locations were nematodes of the genera *Odontophora* and *Paralinhomoes*. In a study by Moreno et al. (2008), *Halalaimus, Molgolaimus, Leptolaimus* were grouped as selective deposit feeder nematodes. Wieser (1953) has also classified these nematodes as selective deposit feeder on the basis of buccal morphology. The genera *Molgolaimus* and *Leptolaimus* were also found to be an important species in O'Brien Bay and Wilkes.

In a microcosm experiment by Millward et al. (2004), it was observed that different metal treatments (Cu, Cd, Cr and Pb) did not produce any significant changes to the

total abundance of copepods. However, they found that in combination with diesel treatments a significant change was elicited, which suggested that the presence of diesel enhanced the effects of metals in sediments. They found that this effect was most pronounced for the metals Cu and Cr. In this current study, it was observed that the mean abundance of copepods in Brown Bay Middle and Wilkes were almost the same as the Control locations, but in Brown Bay Inner, where higher concentration of Cu were observed, the lowest mean abundance occurred. Brown Bay Inner is also known to have higher hydrocarbon concentrations than Brown Bay Middle and Wilkes (Stark et al 2005), due to its close proximity to the old waste disposal site, in addition to the elevated heavy metal levels due to the location. The sensitivity of copepods to copper was also seen in a field study near Chanaral beach (dumping site of Copper mine tailing) of Northern Chile by Lee (2001b). They found that copepods were sensitive to copper pollution and showed a high significant correlation (-0.895, $P < 0.001$) between the mean number of harpacticoid copepods and labile porewater Cu concentration.

The effects of both metal and hydrocarbon contaminations were also observed by Cunningham et al. (2003a) on diatom communities in Brown Bay. Duquesne and Liess (2003) found that the concentrations of Cd, Cr, Pb, Cu and Zn were found to be significantly ($p < 0.01$) higher in Brown Bay than in O'Brien Bay, with Pb and Cu concentrations about 17- and 3.5-fold higher, respectively. They also concluded that these metals originated from the nearby tip site, which is adjacent to Brown Bay. Likewise, Duquesne and Riddle (2002) found that the increase in metal concentrations in the same nearshore environment was due to its position adjacent to the old waste disposal tip site rather than to a natural background enrichment of the area.

It was found in this study that the Wilkes site had a different metal distribution pattern from the other disturbed locations. At this location, the concentration of anthropogenic metals such as Pb and Sn were low. Wilkes, however, has the highest Cd concentrations (2.47 ppm) than other locations. This is 2.40 ppm higher than O'Brien Bay-1, the control location. It is likely that the Wilkes site has had a different contamination history to the other sites. The old waste tip at Wilkes contains variety of metal objects, which includes old machinery, batteries, copper wire and tin cans. These metal objects may have contributed to the different concentrations of Cd and Zn at Wilkes.

McGrady Cove showed some similar meiofaunal distribution patterns to the disturbed locations (Brown Bay) and had similar concentrations of Ag, As, Cd and many of the rare earths. The concentration levels, were however lower than Brown Bay. But for most metals, particularly those most likely to be of anthropogenic origin such as Pb, Sn, Zn, Cu, and Fe, McGrady Cove showed patterns more similar to the control location O'Brien Bay and Wilkes. Concentrations of metals at McGrady Cove may be a combination of the local mineralogy (for rare earths) and long distance transport in Newcomb Bay from contaminated locations such as Brown Bay (for Ag, As, Cd). Heavy metal concentration in sediments from the different locations within the study area mostly showed that contamination decreased with increasing distance from the Casey tip site. Duquesne and Liess (2003), likewise found that the concentration of Co, Cu and Pb in the water column at Casey Station were highest at the tip outlet and decreased as the distance from the tip increased.

Arsenic (As) was also found to be strongly influential on meiofaunal community patterns at Casey. This metal was found at higher concentrations in McGrady Cove (26.00 ppm), Brown Bay Inner (16.21 ppm) and Brown Bay Middle (23.43 ppm). Lower values were found in Wilkes (2.00 ppm), O'Brien Bay-1 (2.00 ppm) and O'Brien Bay-5 (2.00 ppm). The presence of As may be have been contributed by nearby relict penguin colonies as As has been found to be enriched in sediments effected by penguin droppings by about two times above background levels (Yin et al. (2006). This relationship between As and proximity to relict penguin colonies however was not tested in this study. The source of As in the sediments in the current absence of penguins may be attributed to the weathering and erosion of the relict colonies, atmospheric deposition of dust and sediment from the colonies or anthropogenic contribution from nearby sources (Yin et al., 2006).

Although the metal contaminants are present in the marine environment at Casey Station , they are very low by comparison to Australian Environmental Standards (Table 3.5). However, to date there are no guidelines for Antarctica soil and coastal ecosytems.

Table 3.4: Comparison of selected metal/metalloid levels found in this study and Australian Environmental Standard (*Source: National Environment Protection (Assessment of Site Contamination 1999)*)

Metals/Metalloids	Antarctic sediments (this study). Min and max value (in ppm)	Australian Health Investigation Levels (Soil) mg/kg- Standard residential
Arsenic	2.00-45.65	100
Cadmium	0.05-2.58	20
Chromium	0.80-31.16	100
Copper	0.87-50.80	1000
Lead	0.10-0.60	300
Manganese	5.38-17.31	1500
Nickel	0.63-1979	600
Silver	0.01-0.31	No information
Thallium	0.15	No information
Tin	0.38-113.08	No information
Zinc	3.37-53.56	7000

Metals originating from soil erosion have apparently little effect on meiofauna, whereas some metals originating from human activities have a negative correlation towards meiofauna total density (Dalto et al., 2006). These heavy metals have affected the mean abundance of meiofaunal taxa, with lower numbers being were recorded at all disturbed locations. From the results obtained here, it is clear that the lower meiofaunal abundance and/or change in genus composition are linked to the concentration and type of heavy metal pollutants in the sediment. It is however unknown which heavy-metal actually has the greatest influence on the meiofauna community in Casey since most heavy metals were present at all locations. Mahmoudi et al. (2007) demonstrated in a microcosm experiment that contamination with a combination of lead-zinc produced a more diverse nematode communities than those treated by only lead or zinc. This pattern was also observed by Gyedu-Ababio and Baird (2006), where sediment treated with Cu, Fe, Pd, Zn produced a higher total

meiofauna density than those treated with Pb and Zn separately, suggesting that the synergistic effects of the metals may be more influential on the meiofauna communities. Furthermore, Fichet et al. (1999) showed that heavy metal uptake by nematodes and copepods were similar for Cu and Zn, while Cd and Pb were significantly higher in nematodes than copepods.

It is known that several characteristic of meiofauna cause them to respond rapidly to changes in the environment. Previous studies have shown that there is a close relationship between meiofauna and the sediment matrix, thus changes in interstitial chemistry will rapidly lead to alterations in meiofaunal abundance and diversity. This response is particularly rapid in benthic ecosystems with sediments with finer grains sizes. It has been well established that heavy metals are mostly associated with the finer grain size fractions and in this study also it was observed that disturbed locations had finer sediments and higher metal concentrations, while the locations with coarser grain sizes had lower metal concentrations. This study only measured metal concentrations in sediments but hydrocarbon contamination is also known to occur in marine sediments around Casey Station (Deprez et al., 1999, Stark et al., 2005). Studies at Casey station (Stark et al., 2003c, Cunningham et al., 2003b) have shown significant differences in soft sediment assemblages between locations adjacent to an abundant waste dump contaminated by heavy metals and hydrocarbons (Brown Bay) and control locations. Therefore, hydrocarbon contamination is undoubtedly also contributing to patterns of meiofaunal composition as metals and hydrocarbons are frequent co-contaminants in sediments.

Further comparison between findings from this study and other studies are hampered by lack published information on the relationship of meiofauna and metal contaminations in the field. The majority of studies with meiofauna and metals have been conducted in microcosm experiments (Mahmoudi et al., 2007, Ellis et al., 2001, Austen and McEvoy, 1997, Millward and Grant, 2000, Millward et al., 2001, Fichet et al., 1999, Lee et al., 2001b, Ser, 1991, Beyrem et al., 2007, Austen et al., 1994). These laboratory experiments have tended to show that changes in meiofaunal diversity occurs in relation to the response to specific metal contamination. In this field study, such a conclusion could not be made since the effects are combination of various heavy metals and possibly other unmeasured contaminants such as hydrocarbons. In conclusion, this study has demonstrated that the composition of meiofaunal communities near Casey Station is more highly correlated with heavy metals than to grain size. A suite of metals consisting of Ag, Sn, Ba, U, As, Cd, Pb, and Zn were identified as the dominating influence on the meiofaunal communities. This finding supports the suggestion that changes in meiofaunal communities could be use a biomonitors of heavy metals in subtidal ecosystem. A further investigation, is however, needed in order to determine which heavy metals and at what concentration they could affect Antarctic meiofaunal communities.

4.0 THE EFFECTS OF HYDROCARBONS ON MEIOFAUNAL COMMUNITIES

4.1 Introduction

Marine benthic ecosystems are not only subjected to pollution in areas which are highly developed, but pollution is also becoming a threat to the most clean and pristine continent, Antarctica. A small pollution event in Antarctica can have greater significance than occurrences of similar magnitude elsewhere in the world (Clarke and Harris, 2003). The principal forms of human activity in Antarctica are limited to scientific research, fishing, and tourism. The increase in human activities in certain areas of the continent has raised concern about the pollution status and impact to coastal ice-free rocky areas of these activities. Threats from tourism and scientific research are currently small (Clarke and Harris, 2003), and the issue that is of most concern to the benthic communities of Antarctica is the effects of pollution such as heavy metals and hydrocarbons.

4.1.1 Sources and threats of hydrocarbon pollution on benthic ecosystems

Hydrocarbon pollution originates from a variety of different pathways such as shipping and local inputs from research stations (Cripps and Priddle, 1991). There have been a series of oil spills in Antarctica from shipping activities. The biggest oil

spill in Antarctica was in 1989 when an Argentine resupply vessel, *Bahia Paraiso,* with tourists onboard, ran aground in the Bismarck Strait two miles from a scientific research station operated by the United States, near the tip of the Antarctic Peninsula, spilling about 600,000 liters of fuel. In 2001 a Chilean ship, the *Patriarche,* contracted by Ecuador to resupply its Antarctic area station ran aground and spilled petrol off the north-west Antarctic coast. The most recent spill was from the *MS Explorer*, an adventure travel ship which sank in Antarctic waters after hitting an iceberg on November 2007 carrying about 50,000 gallons of diesel, 6,300 gallons of lubricant and 260 gallons of gasoline onboard the vessel. There is also a risk of hydrocarbon spills from station fuel storage and resupply operations although is not considered high. However, in 1990, a spill of 91000 L of special Antarctic blend (SAB) diesel fuel occurred from a fuel storage facility at Casey Station (Deprez et al., 1999).

Petroleum hydrocarbons from oil spills and other sources are a potential threat to the coastal environment. Numerous affects to benthic marine life and the environment can be caused by hydrocarbon pollution, for example, smothering of intertidal and benthic organisms, changes in water temperature, toxicity of different compounds and additives in hydrocarbons, reduced access to food and elevated nutrient concentrations can all modify ecosystems.

To date numerous studies have been conducted to determine the affects of hydrocarbon pollution on benthic ecosystems (Voudrias and Smith, 1986, Cripps and Priddle, 1991, Danovaro et al., 1995, Stark et al., 2003b). Past research has shown that

several areas of the coastal environment around Casey Station have been contaminated with heavy metals and petroleum hydrocarbons (Cunningham et al., 2003). A study by Stark et al. (2003) found that the Casey research station has caused impacts that are detectable in the adjacent marine environment. Pollution sources at Casey Station include the old waste disposal site, the current sewage outfall and fuel spills.

4.1.2 Responses of the benthic community to hydrocarbon pollution

The level of impacts of oil on benthic organisms depends on the concentration of oil, oil type, and the sensitivity of the organisms concerned (Ingole et al., 2006). For example it has been found that, Total Petroleum Hydrocarbon (TPH) has a negative correlation with meiofauna and microfauna abundance but a positive correlation with microbial abundance (Ingole et al., 2006). For example, in a mesocosm experiment conducted by Beyrem et al (2009), sediment treated with mineral oil (Mobil 20 W-50) and synthetic lubricant (Mobil 0 W-40) showed a significant decrease in total nematode abundance (I), species richness (d) and number of species (S) after five days exposure. The effects of hydrocarbon pollution have been found to be more serious for faunal living on the surface of sediment than for those burrowing into the sediments (Cabioch, 1980). It has also been found that sites which have been previously affected by fuel or diesel spill have a low number of copepods and a higher density of nematodes. (Veiga *et al,* (2009).

4.1.2 Meiofauna as an indicator of pollution

Marine benthic fauna and flora have been used as indicators of stress and pollution due to their sensitivity, especially the benthic invertebrates (Calabretta and Oviatt, 2008). They are good indicators of organic pollution due to their constant presence, life cycle, and inactive habit. One of the main components of benthic communities that have been widely used as an indicator is the meiofauna (Kennedy and Jacoby, 1999, Schratzberger et al., 2001, Raghukumar et al., 2001, Beier and Traunspurger, 2001). Meiofauna are an essential component of marine benthic sediment communities, providing ecosystem services such as sediment bioturbation and recycling of organic matter (Higgins and Thiel, 1988). Meiofauna have also been shown to be good indicators of environmental pollution (Moreno et al., 2008). These small and abundant organisms are bound to the sediment throughout their life history and are often sensitive to environmental changes. Other studies have demonstrated that the abundance and diversity of the meiofauna communities are correlated with sediment particle size (Veit-Köhler et al., 2008, Vanhove et al., 2004), food availability (Armenteros et al., 2006) and other environmental parameters (Doulgeraki et al., 2006, Vanhove et al., 1995). The tolerance and occurrence of meiofauna in polluted areas are genera specific but some genera can be classified as indicators. The response of meiofauna is also dependant on the level of contamination (Hedfi et al., 2007). Research has indicated that copepods show more sensitivity towards pollution than nematodes in the meiofaunal community (Moore and Bett, 1989, Warwick, 1986, Sutherland et al., 2007, Lee et al., 2001, Shiells and Anderson, 1985).

Methods using meiofauna to assess the impacts of anthropogenic stressors are not as well developed relative to methods using macrofauna; currently available meiofauna based methods are only applicable in sandy beach habitats experiencing organic enrichment (Coull and Chandler, 1992). Parker (1975) and Raffaelli and Mason (1981) have proposed using the ratio of nematodes to copepods (N/C ratio) as a monitor of pollution or sediment changes that eliminates the need for detailed, time consuming species identification. While, this idea is very attractive it has generated considerable controversy (Warwick, 1981, Lambshead, 1984, Raffaelli and Mason, 1981, Platt et al., 1984, Coull et al., 1981) and it has not been proven to be an accurate predictor of environmental change. While, the technique is simple because no taxonomic experts need be sought, further research is needed to determine the universality of the N/C ratio as a measure of environmental perturbation. Because of rather difficult taxonomy of meiofauna; many perturbation studies prefer not to include them.

To investigate the effects of hydrocarbons on Antarctic meiofaunal communities a manipulative field experiment was undertaken in which four different types of hydrocarbons (three synthetic lubricant oils and diesel fuel treatments) were added to marine sediments. These were deployed in a shallow marine bay and sampled at intervals to determine the effects of hydrocarbons and the response of meiofaunal communities over time.

The aims of the experiment were:

- To examine the effects of four different types of hydrocarbon treatments (Clean lubricant oil, Used lubricant oil, Biodegradable lubricant oil and Special Antarctic Blend Diesel fuel) on sediment meiofaunal communities.
    - To investigate the distribution and diversity of meiofaunal communities in each treatment and at each time.
    - Determine which types of lubricants will most affect the meiofaunal communities
    - Examine how meiofaunal communities change through time

4.2 METHODS

In this study, the environmental variables data; Total Petroleum Hydrocarbon (TPH), grain size and heavy metals data were provided by Human Impact Group, Australian Antarctic Division (AAD) from their SRE4 monitoring project in Casey Antarctica.

4.2.1 Experimental Procedures

Field methods

An experiment was setup in which four different hydrocarbons (SAB diesel fuel, and Clean, Used and Biodegradable lubricant oils) were added to defaunated marine sediments and deployed in trays in a sheltered area in O'Brien Bay (Figure 4.1). The communities colonizing the sediments were monitored for up to five years. Clean sediments were collected from a reference location (O'Brien Bay) and treated with one of four different types of hydrocarbons: a) Biodegradable synthetic lubrication oil (TITAN GTI 1 (0W/20); Fuchs/rapeseed oil), b) Used synthetic lubrication oil (SAE 0W/40; Mobil) – used in CATERPILLAR machine for 150 engine hours, c) Clean (unused) synthetic lubrication oil (SAE 0W/40; Mobil) and d) Special Antarctic Blend (SAB) light diesel fuel. The Mobil SAE0W/40 oil is widely used at Casey and other Australian Stations in power generators and other plant and machinery and the Biodegradable oil is being considered as a possible alternative. In the past used oil was dumped onto the old waste disposal site and is occasionally spilled. SAB diesel is the main fuel used on Antarctic stations to run most plant and machinery and is stored

in large above ground fuel tanks and drums. The site, set-up and sampling of this field experiment have been described in detail previously (Thompson et al., 2006, Powell et al., 2005b).

The experiment was set up at O'Brien Bay with a layout (28 m x 18 m) divided into 24 blocks (3 m x 3 m) that were 2 m apart. Uncontaminated sediment was collected from a different locality to the deployment site in O'Brien Bay. Sediment was sieved through a 500 μm mesh using clean sea water (Thompson et al., 2007). Sediment was collected by divers then transported back to the laboratory for spiking procedure before deployed to the experimental site. Plastic trays made of a $1cm^2$ mesh (tray dimensions 34 cm×23 cm×12 cm) were lined with a 300 μm mesh, and filled with sediment treated with lubricants or diesel. They were deployed carefully to the seabed level at depth of 15 to 18.5m. Trays were positioned on the sediment surface and were in full contact with the sediment on the tray base. The sediment depth in each tray was initially 10 – 11 cm, but after time there was some settling of sediments and total depth of sediment decreased to approximately 8 cm. Five trays were located within each block (i.e. one of each treatment and a control) and trays from four replicate blocks were retrieved at each sampling time. The sediments were sampled for meiofauna at 56 (T2), 102 (T4) and 260 (T5) weeks after deployment. Samples were taken with a small corer (a 60 ml syringe with the end cut off, surface area = 5.599 cm^2) by divers, with two replicate cores per tray. Cores were emptied into 70 ml jars and preserved in 10% formalin.

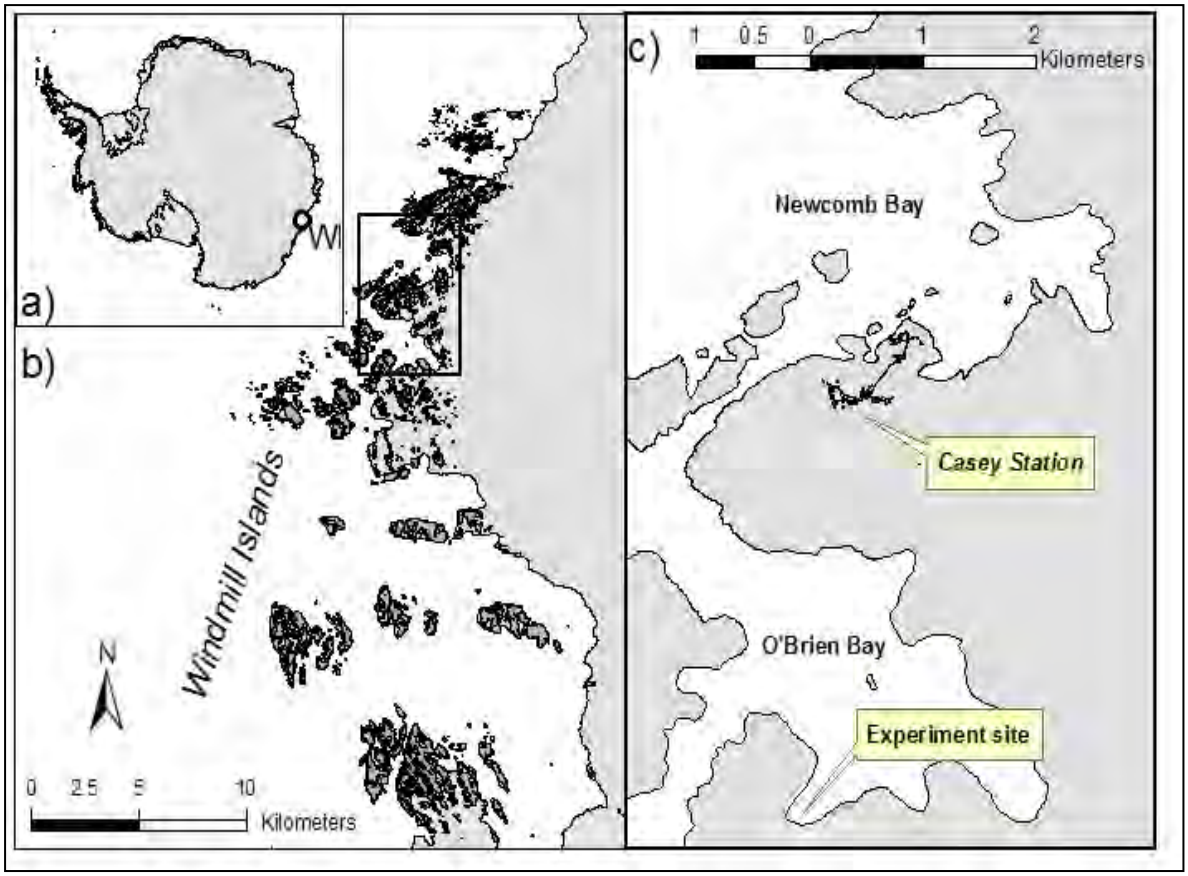

Figure 4.1: Map shows the location of experimental site (O' Brien Bay) in Antarctica.

4.2.2 Meiofauna preparation and identification

The preserved sediment was initially sieved through a 500 µm sieve to remove the coarser sediment fraction. Samples were rinsed thoroughly with tap water to prevent flocculation of Ludox, over a sieve of 32 µm. The sample was transferred from the sieve to a large centrifuge tube. Ludox solution (60% Ludox and 40% water; density = 1.18) was added to tube until the level of mixture was balanced for centrifuging. Then the sample was centrifuged at 2800 rpm for 10 min. The supernatant was decanted and collected and the remaining sediment pellet was resuspended. This process was repeated three times.

All supernatants were filtered through a 100 µm sieve, followed by a 32 µm sieve. The supernatant was finally rinsed over a 32 µm sieve with tap water for some time because Ludox and formalin will react and form a white gel that is difficult to wash out. After the extraction, 4% formaldehyde was added again to the treated sample. The organisms retained by the 32 µm sieve were fixed in 4% formaldehyde. The counting process was facilitated by staining of the entire sample with 1% of Rose Bengal.

All animals retained on a 32 µm sieve were counted and sorted into major taxa. The major taxa (Nematoda and Copepoda) were counted using a dissecting microscope at 25x magnification (ZEISS Stemi 2000; Zeiss Inc., Germany). Per sample, 200 nematodes (or all nematodes when density less than 200 individuals.) were picked out

at random and mounted on slides in glycerine after a slow evaporation procedure (modified after Riemann, 1988) for identification to genus level mainly using Platt and Warwick (1983, 1988) and NeMys online identification (Deprez et al., 2005). All copepods were picked out and mounted on slides in glycerine without evaporation for identification to family level using THAO: Taxonomische Harpacticoida Archiv Oldenburg (2005), Huys et al. (1996) and Bodin (1997). The identification of nematodes and copepods were done using 1000 times magnification.

4.2.3 Total Petroleum Hydrocarbon Analysis

A homogenized sub sample of approximately 5 g of sediment from the top 1 cm slice from each core was extracted overnight with 10 ml of deionized water, 9 ml of dichloromethane and 1 ml of dichloromethane containing as internal standards (IS) 1 mg l^{-1} of cyclooctane, 1 mg l^{-1} of bromoeicosane, 0.25 mg l^{-1} of 1,4-dichlorobenzene, 0.25 mg l^{-1} of deuterated tetracosane and 0.1mg l^{-1} *p*-terphenyl. The samples were centrifuged for 10 mins at 3,000 rpm at 10˚C and the supernatant removed to a clean vial. The supernatants were analyzed for total petroleum hydrocarbons (TPH) by gas chromatography-flame-ionization detector (GC-FID) on a Varian CP3800 (Varian) with a BP-1 (SGE) column (25 m; i. d. 0.22 mm; 0.25 μm film) and a Varian 8400 autosampler controlled with Star Chromatography Workstation. One microliter of extract was injected (1:20 split using a 1079 PTV injector) at 1.3 ml min^{-1}. The detector flow rates were 29 ml min^{-1} for nitrogen, 30 ml min^{-1} for hydrogen and 300 ml min^{-1} for air. The oven temperature was initially 40˚C held for 3 mins, then increased at a rate of 18˚C min^{-1} to 330˚C and held for a total run-time of 23 mins.

The TPH concentrations were determined using calibration curves based on standards prepared with each of the oils at concentrations of approximately 8000, 5000, 2500, 1000, 500, 200, 100, 50 and 0 mg kg^{-1}. Using the IS response relative to the total detector response to all hydrocarbons, the TPH: IS ratio could be measured. This ratio was also calculated for the samples and used to determine the TPH values.

4.2.4 Statistical methods

Univariate analysis

A two factor experimental design was used to analyse abundance data (by ANOVA) and community data (by PERMANOVA), with Time (3 times) and Treatment (5 treatments) as orthogonal factors. Cochran's test was used to test for homogeneity of variances in ANOVAs. If variances were heterogeneous, data were transformed (Snedecor and Cochran, 1980, Underwood, 1981). Where significant heterogeneity of variances could not be removed by transformation, a lower significance level of P = 0.01 was used. When a significant effects were encountered, post-hoc multiple comparisons among means using the Student–Newman–Keuls (SNK) test (at a=0.05) were carried out.

Multivariate analysis

Multivariate analyses of community composition were undertaken using non-metric multi dimensional scaling (nMDS), Analysis of Similarities (ANOSIM) and similarity percentages (SIMPER) procedures using the PRIMER v6.0 statistical software

package (Clarke and Gorley, 2006) and PRIMER + PERMANOVA (Anderson et al., 2007). Stress values in nMDS provide a measure of goodness of fit for the ordination with values ranging from 0 to1 (Clarke, 1993). Values close to 0 indicate a good fit whereas a stress value greater than 0.3 is no better than arbitrary (Clarke, 1993). The Bray-Curtis distance measure was used to determine similarities between samples, after square-root transformation of abundance data.

PERMANOVA and one-way ANOSIM was performed to determine whether there were significant differences among groups and to compare the similarities in the composition of meiofaunal communities from six locations. Pairwise R-values give an absolute measure of how separate groups are on a scale of 0 (indistinguishable) to 1 (all similarities within groups are less than any similarity between groups).

SIMPER analyses were used to determine which taxa were responsible for compositional differences observed between meiofaunal communities. Clarke and Warwick (1994) stated that as a guideline, species which have a SIMPER ratio greater than 1.3 are likely to be useful for discriminating between groups.

Data transformation was applied in analyses to validate statistical assumption for parametric techniques and to weight the contributions of common and rare taxa in the multivariate representations (Clarke and Warwick, 2001). The transformations play an important role to balance between contributions from common and rarer family/genus in the measure of similarity of two samples (Clarke and Warwick, 2001). The square-root transformation has a similar effect in reducing the weighting of abundant

family/genera but has the advantage that, when similarity is assessed by the Bray-Curtis measure, the similarity coefficient is invariant to a scale change (Field et al., 1982). A square-root transform was applied to the community composition and nematode abundance data as this study not have high abundances of a single species (rarely had abundances over 30 of any taxa). The log transformation has the powerful effect of 'scaling down' very abundant family/genus and thus increasing equitability of the datasetLog transformation was applied copepod abundance data because to down weight contributions from both common and rare taxa.

4.3 RESULTS

4.3.1 Lubricant contamination

The Total Petroleum Hydrocarbon (TPH) concentrations (mg/kg) in the top 1 cm of sediments contaminated with Clean, Used, Biodegradable, and SAB diesel decreased from 0 weeks to 260 weeks (Figure 4.2a). In the Control, TPH increased after the experiment started and decreased after 56 weeks and again increased slightly after 104 weeks (Figure 4.2a and 4.2b). The maximum TPH concentrations in Control sediments after deployment were less than 73 mg/kg. In this study, the highest TPH recorded in T2 for all four hydrocarbon treatments was in Biodegradable (5166 mg/kg) while the lowest was found in SAB diesel (1046 mg/kg). After 260 weeks, the TPH concentrations in all treatment were still high compared to the Control. The highest TPH concentration after 260 weeks was in Used lubricant treatment which the value of 1046 mg/kg.

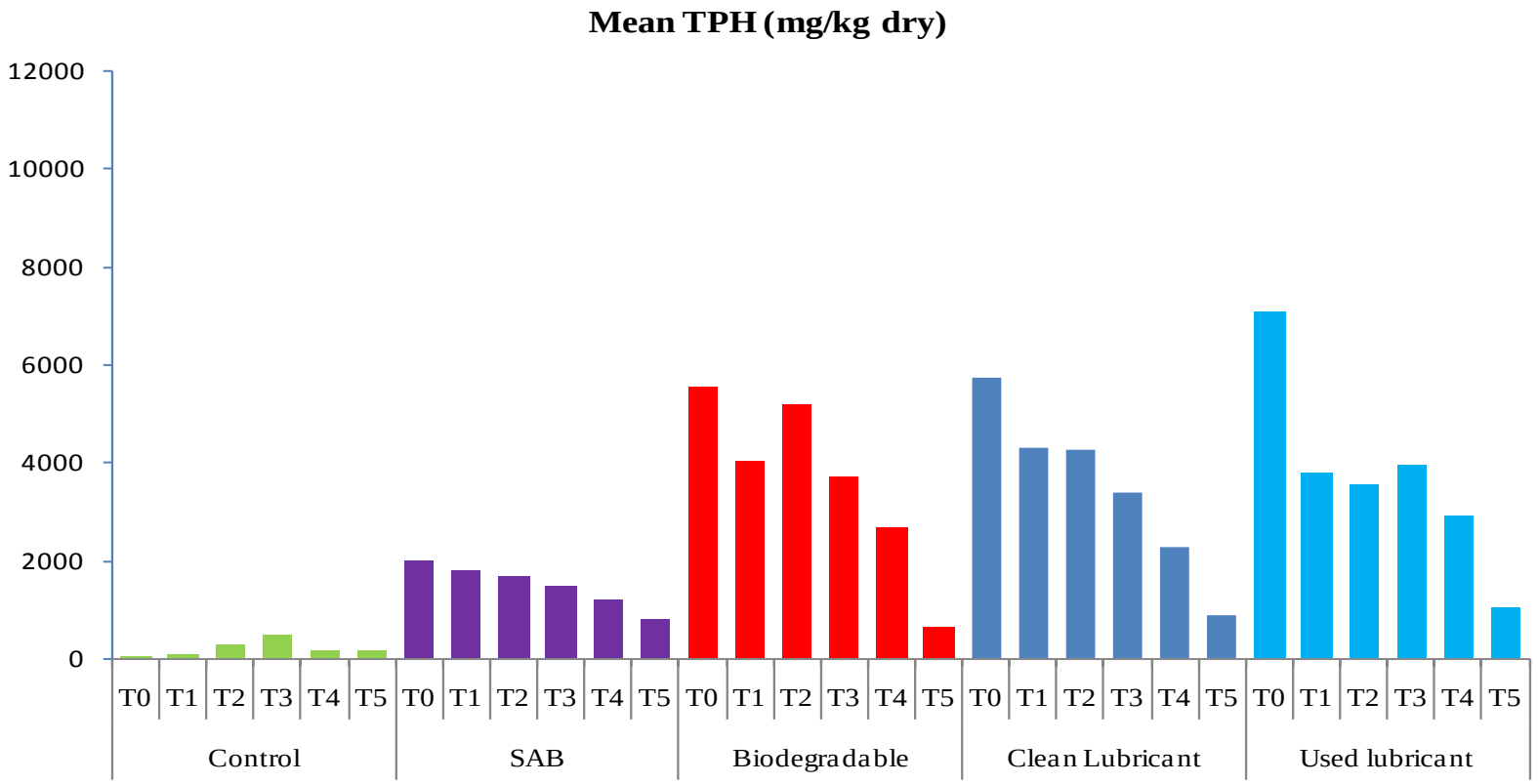

Figure 4.2a: Total petroleum hydrocarbon (mg/kg) concentrations in the top 1cm of sediments treatment; Control, Biodegradable, Clean, Used and SAB diesel at 0, 5, 56, 65, 106 and 260 weeks. (*Source: Human Impact Program, Australian Antarctic Division 2009*)

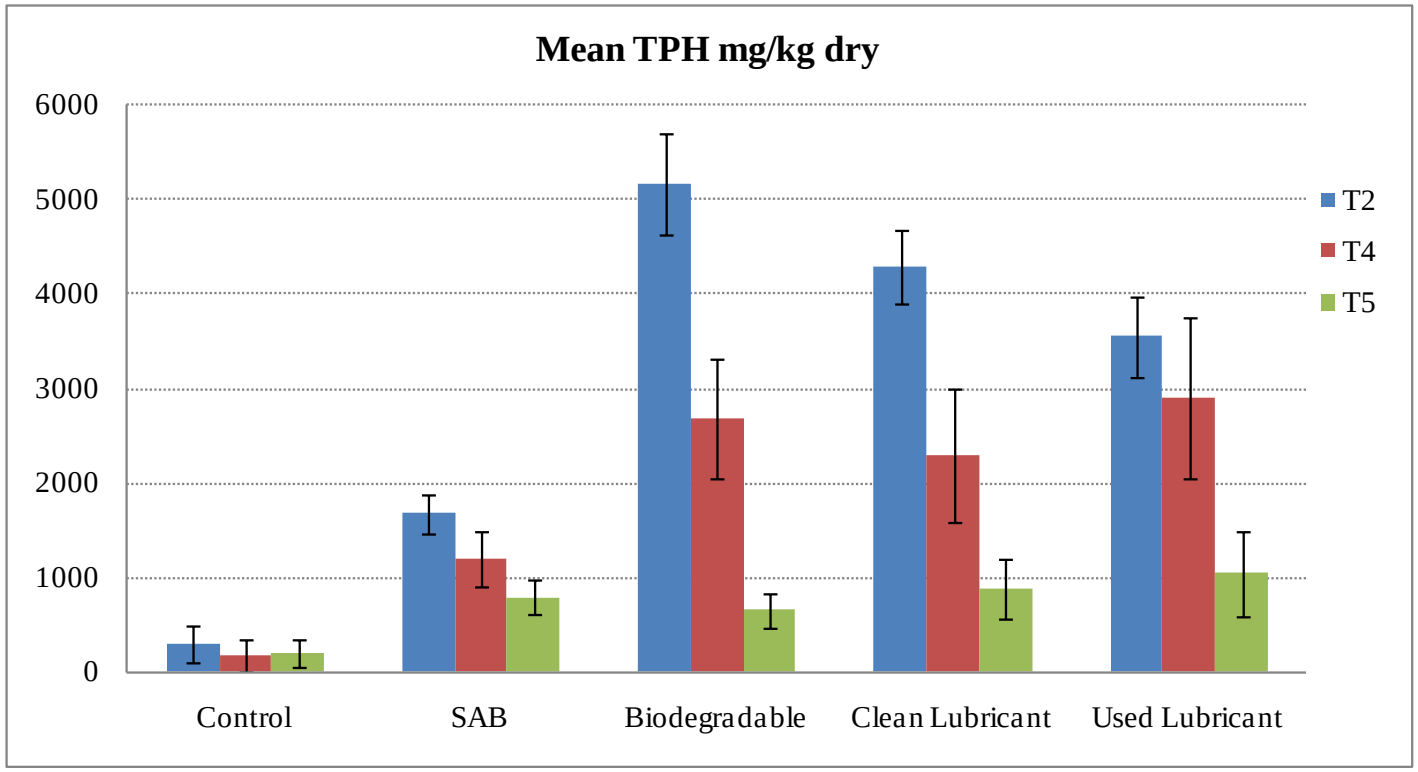

Figure 4.2b: Total petroleum hydrocarbon (mg/kg) concentrations in the top 1cm of sediments treatment; Control, Biodegradable, Clean, Used and SAB diesel in this study at 56, 106 and 260 weeks.

4.3.2 Composition of the meiofaunal

A total of 10,633 individuals from 65 higher meiofauna taxa comprised of 40 genera of nematodes and 25 families of harpacticoid copepods were identified in 120 samples from five treatments (Control, Clean lubricant oil, Used lubricant oil, Biodegradable lubricant oil and SAB diesel) over 260 weeks experiment time (T2, T4 and T5). These hydrocarbon treatments had a strong impact on meiofaunal community, affecting both nematodes and copepods composition (Table 4.1). The highest abundances were recorded in the Control treatment (average density ranges from 3811 to 8209 ind. 10 cm^{-2}). Lowest value were observed in the SAB (2251 to 4453 ind. 10 cm^{-2}).

There was significantly difference between time and treatments in Shannon–Wiener diversity (*H*') (ANOVA, $P < 0.01$), species richness (Margalef's *d*) (ANOVA, $P < 0.01$) and evenness (Pielou's *J*') (ANOVA, $P < 0.01$). All indexes were lower in T2 in most treatments and they gradually increase as duration increases (Figure 4.3).

Table 4.1: PERMANOVA and ANOVA results for meiofaunal communities in Antarctic hydrocarbon treatment experiment.

Source	df	MS	F	P
*Community composition**				
Treatment	4	6369.7	16.69	0.0001
Time	2	16847	44.142	0.0001
Treatment x Time	8	3870.6	10.142	0.0001
Residual	105	381.65		
*Nematode Abundance**				
Treatment	4	1124.18	1287.71	< 0.0001
Time	2	3.6629	4.2	0.0177
Treatment x Time	8	4.9246	5.64	< 0.0001
Residual	105	0.873		
Copepod Abundance[#]				
Treatment	4	1.3198	35.76	< 0.0001
Time	2	0.7618	20.64	< 0.0001
Treatment x Time	8	0.3119	8.45	< 0.0001
Residual	105	0.0369		
Nematodes Taxa[†]				
Treatment	4	40.6958	3.93	0.0052
Time	2	1045.43	100.92	< 0.0001
Treatment x Time	8	54.1208	5.22	< 0.0001
Residual	105	10.3595		
Copepod taxa[†]				
Treatment	4	59.633	19.1	< 0.0001
Time	2	12.9	4.13	0.0187
Treatment x Time	8	30.515	9.77	< 0.0001
Residual	105	3.1226		

[†]Data Untransformed; *Data Square-root transformed; [#]Data Log transformed

Figure 4.3: Mean abundances (+SE) Shannon–Wiener diversity (*H*' log *e*), species richness (Margalef's *d*) and evenness (Pielou's *J*').

There was a significant effect of hydrocarbon treatments on meiofaunal mean abundance, largely due to nematodes, which comprised the bulk of the community. The highest mean abundance of meiofauna was in Control and Biodegradable treatments, while SAB treatments showed lowest mean abundance (Figure 4.4a). A comparison of time exposure among treatments showed that the mean abundance of meiofauna in each treatment changed very little over time, and was significantly greater in the Control and Biodegradable treatments, which were not significantly different (Figure 4.4b). Mean abundances were significantly lower in clean, used and SAB treatments at all times (Figure 4.4b). A clear trend was observed in SAB Lubricant from T2 to T5, with a small but significant decline in mean total abundance of meiofauna, which was largely due to a decline in nematode abundance (Figure 4.3d). The ANOVA results indicate no significant difference in Control and Biodegradable in the mean abundance of nematodes (Table 4.2). Nematode diversity (no. of genera) increased from T2 to T5 in all treatments (Figure 4.4b) (ANOVA, $P < 0.001$; Table 4.1 and 4.2). An increase in the number of genera of 25% to 50% was seen in treatments over time (Figure 4.4b).

Copepods showed a very different response to hydrocarbon treatments than the nematodes. Diversity and abundance were greater in the Control than the other treatments at T2 and T5, but with a very different pattern at T4, with greatest diversity and abundance in the Used treatment (Figure 4.4d, Table 4.2). The mean abundance of copepods was highest at T4 in all the hydrocarbon treatments, when there was a significant increase, followed by a decrease to T5 (Figure 4.4e). This pattern was not observed in the control.

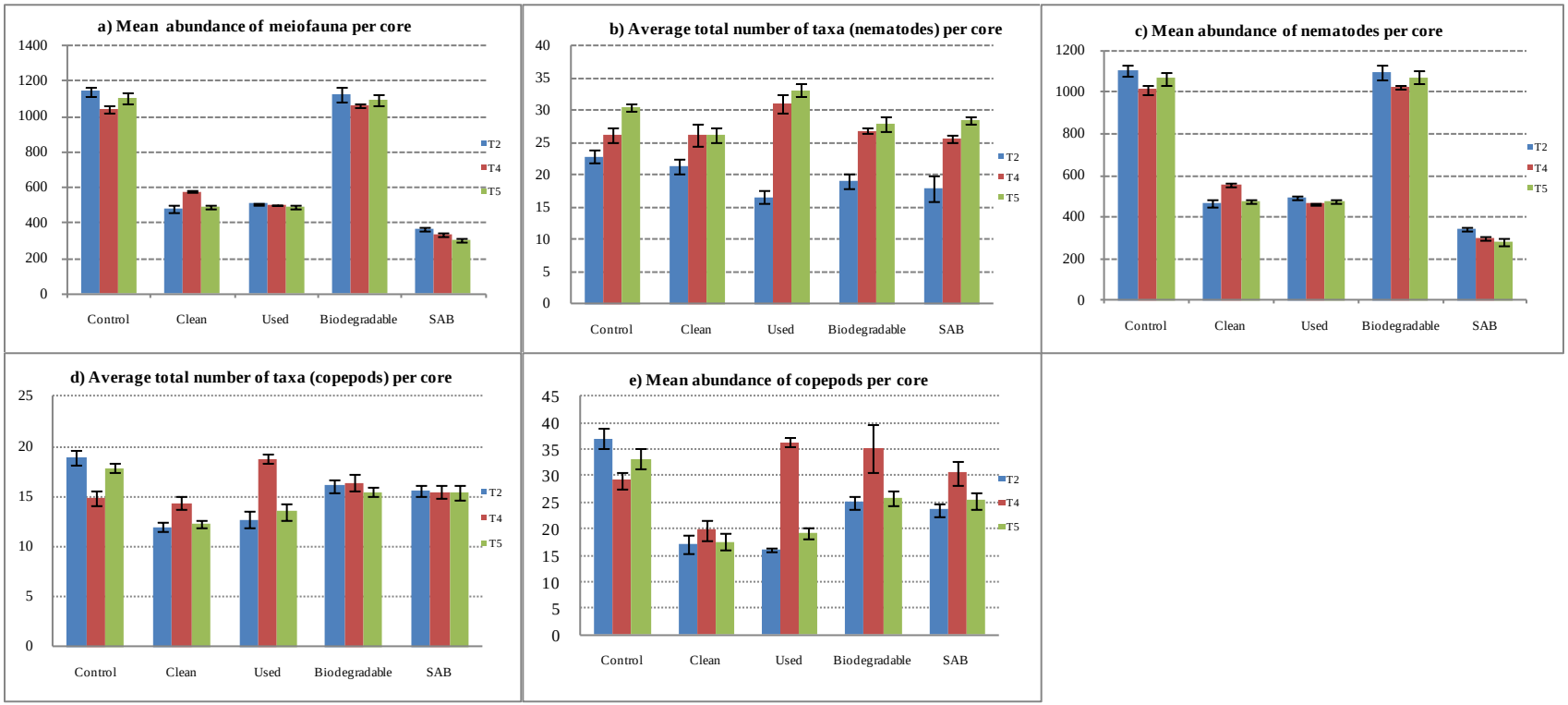

Figure 4.4a: Mean abundance meiofaunal and numbers of taxa found in hydrocarbon treatment experiment in Casey.

Table 4.2: Results of SNK tests for significant factors in ANOVAs of total meiofaunal taxa and abundances in hydrocarbon contaminated sediment.

Factors		Nematode Taxa	Nematode Abundance	Copepod Taxa	Copepod Abundance
Treatment		Used = Control = Clean = Bio = SAB	Bio = Control > Clean = Used > SAB	Control > Bio = SAB = Used > Clean	Control > Bio = SAB > Used > Clean
Time		T5 > T4 > T2	T2 = T4 = T5	T4 > T2 = T5	T4 > T5 = T2
Treatment x Time	T2	Control = Clean = Bio = SAB = Used	Control = Bio > Used = Clean = SAB	Control > Bio = SAB > Used = Clean	Control > Bio = SAB > Clean = Used
	T4	Used > Bio = Control = Clean = SAB	Bio = Control > Clean > Used > SAB	Used > Bio = SAB = Control = Clean	Used = Bio = SAB = Control > Clean
	T5	Used = Control = SAB = Bio = Clean	Bio = Control > Clean = Used > SAB	Control > Bio = SAB = Used = Clean	Control > Bio = SAB > Used = Clean
Time x Treatment	Control	T5 > T4 > T2	T2 =T5 = T4	T2 =T5 >T4	T2 = T5 = T4
	Clean	T5 = T4 > T2	T2 =T5 = T4	T4 > T5 = T2	T4 = T5 = T2
	Used	T5 = T4 > T2	T2 =T5 = T4	T4 > T5 = T2	T4 > T5 = T2
	Bio	T5 = T4 > T2	T2 =T5 = T4	T4 = T2 = T5	T4 > T5 = T2
	SAB	T5 = T4 > T2	T2 =T4 = T5	T2 =T4 = T5	T4 = T5 = T2

4.3.4 Multivariate analysis of assemblages

Multivariate analysis of assemblage composition revealed significant differences between treatments at all times. The nMDS ordination of all 120 samples in the hydrocarbon experiment showed distinct separation of treatments in the meiofaunal communities at T2, T4 and T5 (Figure 4.5a). All treatments were significantly different and can be seen to form independent groups except Control and Biodegradable, where there was some overlap during T2 and T4 (Figure 4.5b). At T5, all treatments showed independent groups (Figure 4.5b). Results showed the variation within treatments was less than the variation between treatments. This is supported by one way ANOSIM analysis in Table 4.3. It is clear that each treatment elicited a different response in the meiofaunal communities, as evidenced by their clear separation, but in different directions from the Control treatment in each nMDS (Figure 4.5b).

A one-way ANOSIM of the four treatments groups against the Control treatment indicated significant differences ($p > 0.1$) for all pairwise comparisons, with a range of R-values indicating differing degrees of discrimination (Table 4.3). Pairwise comparisons of Control and Biodegradable treatment produced the smallest R value during all times (T2, T4 and T5) indicating that these two treatments had the most similar composition of meiofaunal communities.

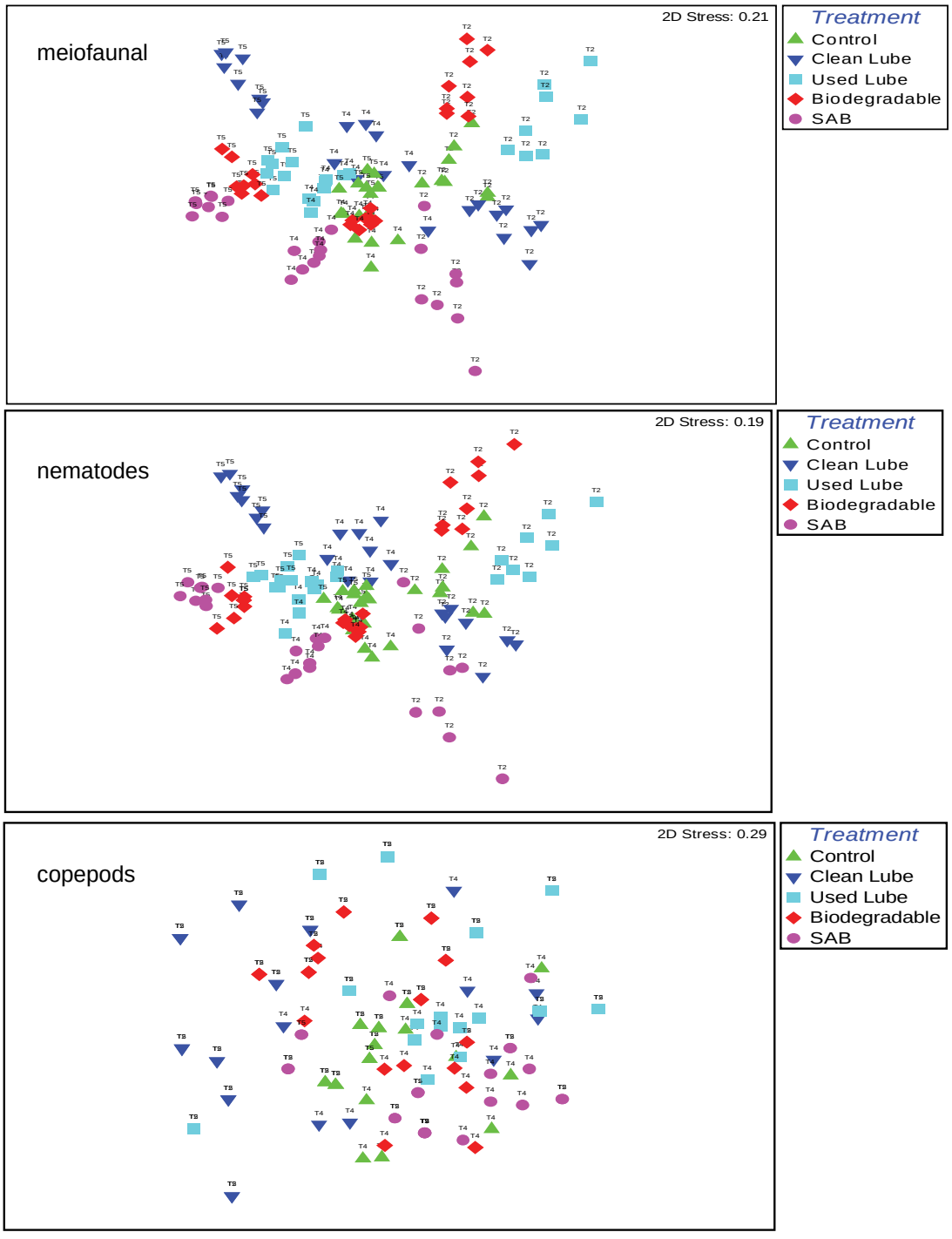

Figure 4.5a: nMDS representing the meiofaunal community, nematodes only and copepods only at all time (T2, T4, and T5) based on square root transformed abundances and Bray-Curtis similarities.

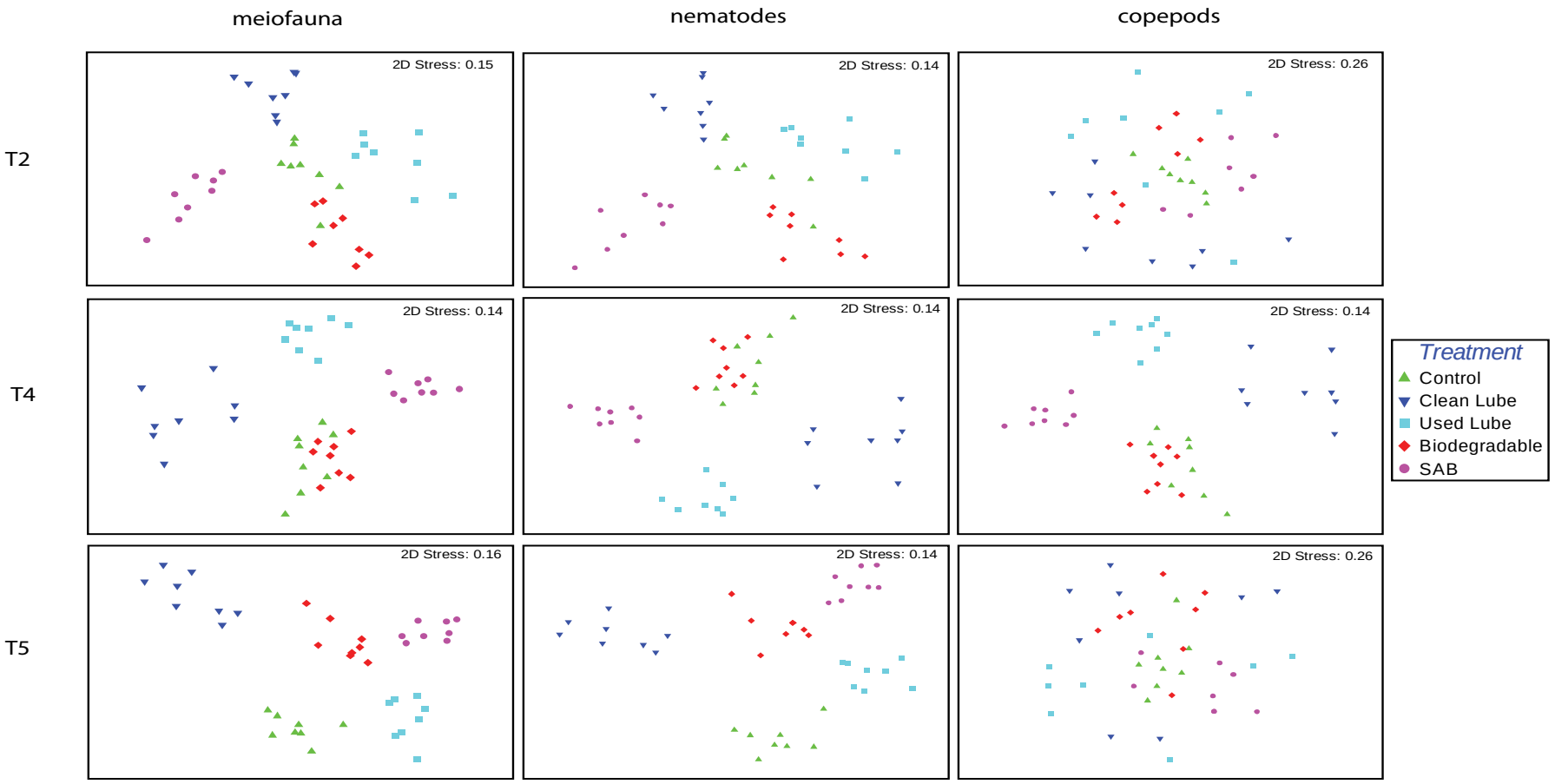

Figure 4.5b: nMDS representing the meiofaunal community, nematodes only and copepods only at T2 (56 weeks), T4 (104 weeks) and T5 (260 weeks). Based on square root transformed abundances and Bray-Curtis similarities.

Table 4.3: ANOSIM shows comparison of meiofaunal community structure between the Control and the treatments at all time interval.

T2, Global R= 0.869, P=0.1					
Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >=Observed
Control, Clean Lube	0.795	0.1	6435	999	0
Control, Used Lube	0.757	0.1	6435	999	0
Control, Biodegradable	0.655	0.1	6435	999	0
Control, SAB	0.871	0.1	6435	999	0

T4 Global R= 0.893, P=0.1					
Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >=Observed
Control, Clean Lube	0.926	0.1	6435	999	0
Control, Used Lube	0.955	0.1	6435	999	0
Control, Biodegradable	0.357	0.1	6435	999	0
Control, SAB	0.97	0.1	6435	999	0

T5 Global R=0.983, P=0.1					
Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >=Observed
Control, Clean Lube	1	0.1	6435	999	0
Control, Used Lube	0.984	0.1	6435	999	0
Control, Biodegradable	0.984	0.1	6435	999	0
Control, SAB	1	0.1	6435	999	0

4.3.5 Interactions of different Treatments and Times

PERMANOVA results showed significant effects of treatments, time, and an interaction between treatment and time (PERMANOVA, $P < 0.001$; Table 4.4). The treatments were all significantly different to each other at all times and the interaction related mainly to the size of the differences and relationships among hydrocarbon treatments. Importantly, the Control maintained significant differences with treatments at all times, though the size of the effect varied. The Control showed the least degree of change between all time comparisons, excluding the Used oil treatment which changed the least between T4 and T5 (Table 4.5b). The Control has the highest percentage of similarity (63.5%) between times (Table 4.5b).

Table 4.6a and 4.6b show the percentage similarity (from PERMANOVA) breakdown of the interaction term between treatment and time. Among the treatments, Control and Biodegradable were the most similar over time (Table 4.6b). All treatments become less variable over time (Table 4.6b). At all times, the Biodegradable is the most similar to Control but at the end of experiment, it was more different to the Control than at T4 (Figure 4.6a).

SAB lubricant initially had the largest effect but by T5 all treatments were of approximately the same magnitude of difference from the Control (Table 4.6b and Figure 4.6b).

Table 4.4: PERMANOVA two-factors results.

Source	df	Pseudo-F	P(perm)	Unique perms
Treatment	4	16.69	0.0001	9871
Time	2	44.142	0.0001	9909
Treatment x Time	8	10.142	0.0001	9805
Residual	105			
Total	119			

Estimates of components of variation

Source	Estimate	Square root
S(Treatment)	16.87	15.80
S(Time)	27.83	20.29
S(Treatment x Time)	29.49	20.88
V(Residual)	25.81	19.54

Table 4.5a: Pairwise test of Time within Treatment.

Groups	Control		Clean		Used		Biodegradable		SAB	
	t	P	t	P	t	P	t	P	t	P
T2, T4	3.1768	0.0001	3.6605	0.0002	4.1823	0.0001	4.1403	0.0002	4.0893	0.0004
T2, T5	2.8090	0.0002	5.6411	0.0003	4.4828	0.0003	4.5433	0.0001	4.9671	0.0002
T4, T5	3.4956	0.0001	4.4435	0.0004	2.4704	0.0004	3.6477	0.0002	4.6212	0.0001

Table 4.5b: PERMANOVA interaction treatment and time (Times within Treatment).

Percentage of similarity between times.

	Control	**Clean**	**Used**	**Biodegradable**	**SAB**
T2, T4	58.9	53.7	48.2	50.4	51.0
T2, T5	63.5	41.7	44.7	44.7	42.7
T4, T5	61.0	52.6	66.7	59.9	56.4

Table 4.6a: Pairwise test of treatment vs control within level T2, T4, and T5 of factor 'Time'.

Groups	T2		T4		T5	
	t	P	t	P	t	P
Control, Clean Lube	2.9709	0.0002	2.8200	0.0004	5.0203	0.0001
Control, Used Lube	2.6703	0.0002	3.0632	0.0001	3.5203	0.0005
Control, Biodegradable	2.3929	0.0005	1.6227	0.0006	3.1465	0.0001
Control, SAB	3.0125	0.0002	3.2198	0.0002	4.4128	0.0001

Table 4.6b: PERMANOVA interaction treatment and time (Treatment within Times).

Percentage of similarity **between** treatment			
	T2	**T4**	**T5**
Control vs Clean	58.9	62.4	52.9
Control vs Used	58.4	62.8	60.5
Control vs Biodegradable	60.8	72.6	63.2
Control vs SAB	55.5	63.8	56.7
Percentage of similarity **within** treatment			
	T2	**T4**	**T5**
Control	70.7	73.7	76.4
Clean	71.1	71.6	77.1
Used	67.6	74.7	73.6
Biodegradable	67.8	76.5	73.8
SAB	67.2	77.6	76.5

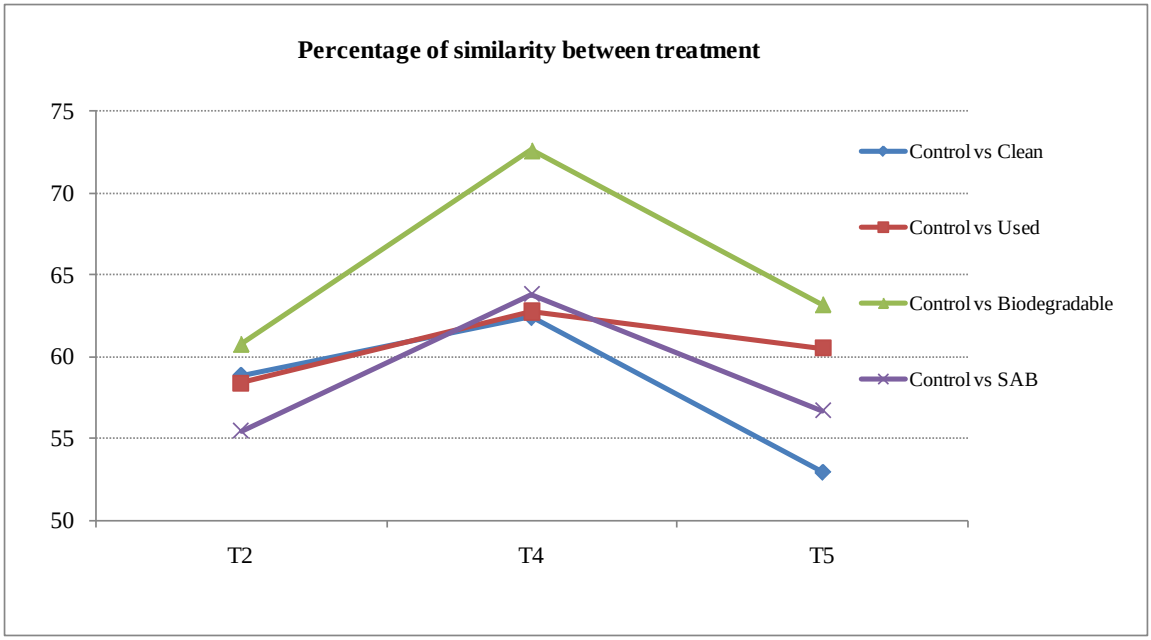

Figure 4.6a: Results show the percentage similarity between treatment times in the interaction of treatment and time.

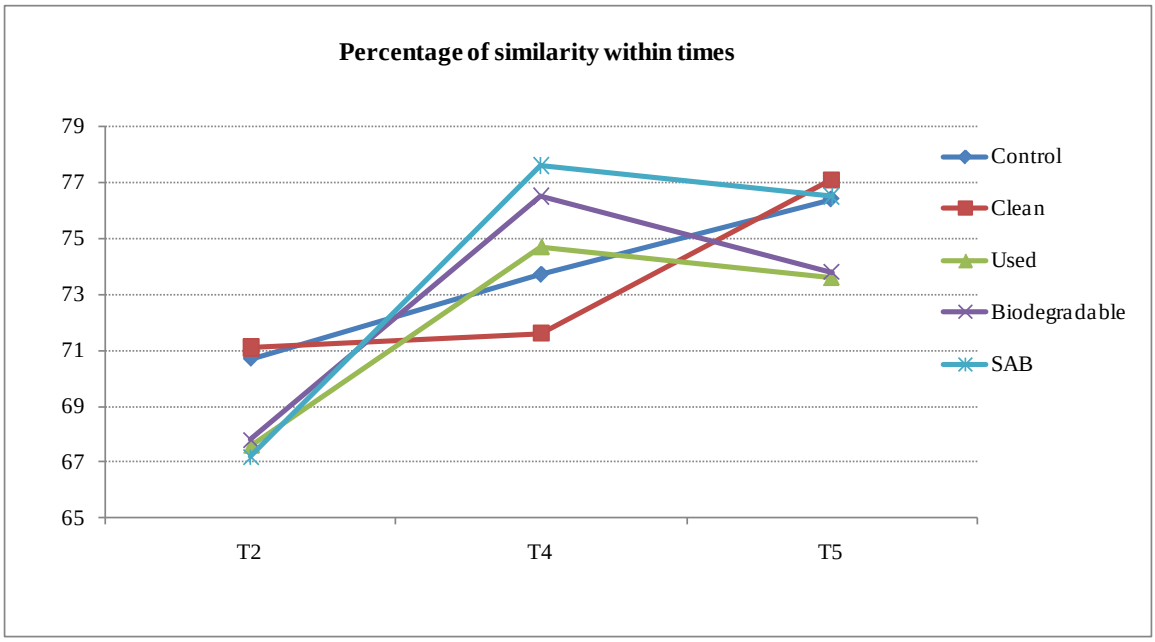

Figure 4.6b: Results show the percentage similarity within times in the interaction of treatment and time.

Similarity percentage analysis (SIMPER) was used to determine the taxa contributing to the observed difference between and within treatments. The results of the SIMPER analyses are shown in Table 4.7, 4.8 and 4.9. Several taxa contributed strongly to differences between treatments at all times, including *Daptonema*, *Dichromadora*, *Neochromadora*, *Paracanthonchus*, *Paramesonchium*, *Halalaimus*, *Linhomoeus* and *Monhystera*. Nematodes contributed the most to the dissimilarity between treatments and times but harpacticoid copepods contributed most to the similarity within treatments and times.

Compared to spatial study in Chapter 2, the control treatments in the hydrocarbon experiment have a greater number of taxa (65 out of 70) than the spatial study (O'Brien Bay-1 and O'Brien Bay-5) which is 55 taxa out of 70 (Figure 4.7). Five taxa, *Bolbolaimus*, *Desmorella*, Ancorabolidae, Dactyloposiidae and Romatidae were not found in the control treatments of hydrocarbon experiment.

Differences between the Control and the treatments were due to a range of different fauna being present at different times, with some contrasting relationships between each of the hydrocarbon treatments and the Control. One similarity between the Control and the treatments was the reduction in the abundance of many taxa from T2 to T5. For some taxa, a higher abundance was observed at T2 (Figure 4.8). For example, at T2 the abundance of *Neochromadora* in the Used and Biodegradable oil treatments was greater than in the Control, but was lower than the control in the Clean and SAB treatments.

Neochromadora was also clearly an opportunistic early colonizer with initially large abundances that were reduced in all treatments and Control by T4 and T5. Used lubricant treatment was characterized by the high occurrence of *Neochromadora* and *Dichromadora*, which were also found in Biodegradable treatments at T2. The major difference between these two treatments was the absence of *Halalaimus* at T4 and T5 in Biodegradable and *Linhomoeus* at T2 in Used treatments.

In the Clean oil treatments, *Daptonema* and *Monhystera* was the earliest opportunistic taxa at T2, this taxa however decreased by T4, whereas *Paramesonchium* and *Linhomoeus* increased in abundance by T5. The Clean oil treatments treatment showed a resemblance to the Control in the abundance of *Neochromadora* and *Paracanthonchus* at T5. Low values were obtained for both taxa.

Some hydrocarbons initially acted as a stimulus for recruitment, for example *Paracanthonchus* had low abundances in all treatments at T2 except for the SAB treatment, which had high abundances in the SAB treatment, but there was less difference between treatments and Control by T4 and T5. *Halaimus* was initially present in all treatments but in the SAB and biodegradable treatments it was absent at T4 and T5.

BVSTEP procedures were conducted to determine the best subsets of variables (taxa) that could “best” match that of the treatments data, i.e., could explain the most variation in

similarity or dissimilarity between treatments with respect to meiofaunal communities. A group of 16 taxa, from a total of 65 (*Chromadora, Chromadorella, Draconema, Halalaimus, Leptolaimus, Monhystera, Neochromadora, Southerneilla, Theristus, Trefusia, Vasostoma,* Ameridae, Cletodidae, Idyanthidae, Pseudotachidiidae and Tachididae) were the best subset that could explain most variation in the meiofaunal composition pattern (Spearman correlation coefficient $\rho = 0.952$, $P < 0.001$). Figure 4.9 shows the mean abundances of these taxa. *Halalaimus*, *Monhystera* and *Neochromadora* were among the most distinctive taxa in terms of response to the treatments.

Table 4.7: Taxa responsible for difference between hydrocarbon treatments in T2 based on SIMPER analysis of square-root transformed data.

T2	Control (78.51)		Clean Lube (73.09)		Used Lube (72.33)		Biodegradable (74.83)		SAB (71.02)	
Taxa	Av.Abund	Contrib %	Av.Abund	Contrib %	Av.Abund	Contrib %	Av.Abund	Contrib %	Av.Abund	Contrib %
Dichromadora	5.61	6.98			5.75	11.24	5.65	9.55	3.51	5.20
Neochromadora	5.28	6.22			7.34	13.48	8.12	13.51		
Monhystera	4.53	5.16	6.80	12.36	4.72	6.68	2.49	3.31		
Daptonema	3.85	4.44	5.54	9.91					3.04	5.21
Chromadorita	2.86	3.85			2.51	4.60	2.42	3.70	3.06	5.00
Ascolaimus	2.70	3.44								
Chromadorina	2.23	2.98	2.69	4.41	2.45	4.63				
Paracanthonchus	2.22	2.95							8.30	14.18
Halalaimus	3.04	2.57	4.21	6.48					5.00	8.89
Odonthopora	2.33	2.54	2.54	4.22						
Chromadorella	1.95	2.51			3.79	7.38				
Linhomoeus	1.82	2.33					4.48	6.91		
Metalinhomoeus	1.86	2.31							3.07	5.03
Laophontidae	1.72	2.31								
Sphaerolaimus			3.03	5.25	2.66	5.24				
Chromadora			2.67	4.33						
Theristus			2.57	4.28						
Gammanema							3.11	4.63		
Promonhystera							2.40	3.84		
Spirobolbolaimus							2.41	3.40		
Paralinhomoeus							1.82	2.92	3.23	5.45
Leptolaimus									2.35	3.71
Total Taxa : Accumulative %	14	50.6	8	51.23	7	53.26	9	51.77	8	52.66

Table 4.8: Taxa responsible for difference between hydrocarbon treatments in T4 based on SIMPER analysis of square-root transformed data.

T4	Control (79.23)		Clean Lube (78.52)		Used Lube (79.72)		Biodegradable (81.02)		SAB (83.65)	
Taxa	Av.Abund	Contrib%	Av.Abund	Contrib%	Av.Abund	Contrib%	Av.Abund	Contrib%	Av.Abund	Contrib%
Dichromadora	4.67	6.07	4.91	6.4	2.07	2.25	4.7	5.53	2.39	3.01
Monhystera	4.47	5.46	4.03	5.1	4.72	5.52	3.47	4.12		
Daptonema	3.6	4.78	2.77	3.67			2.69	3.17	5.06	6.44
Paralinhomoeus	3.89	4.67	4.29	5.83			4.72	5.94		
Paramesonchium	3.92	4.61			3.31	3.51	4.13	4.87		
Molgolaimus	3.69	4.53					3.54	3.97	2.08	2.48
Chromadorita	3.19	4.05	3.5	4.72			2.55	3.01		
Leptolaimus	3.38	3.89	3.18	4.22	4.34	4.55			5.54	7.05
Desmolaimus	3.2	3.85	3.28	4.45			2.83	3.23		
Linhomoeus	2.62	3.06			3.21	3.54	3.4	4.18	3.52	4.32
Sabatiera	2.29	3.05								
Chromadorina	2.28	2.67	3.84	4.99	2.44	2.62			3.09	3.3
Halalaimus			3.39	4.52						
Gammanema			2.88	3.87						
Sphaerolaimus			2.23	2.85						
Ascolaimus					2.75	2.86				
Paramonhystera					2.68	2.73	2.8	3.54	2.8	3.1
Trefusia					2.4	2.64				
Desmodora					2.47	2.62			2.31	2.89
Neochromadora					2.38	2.61				
Chromadorella					2.49	2.47				
Chromadora					2.43	2.44				
Odonthopora					2.18	2.34				
Rhabdocoma					2.26	2.3			2.1	2.51
Paracanthonchus					2.21	2.27	2.2	2.69	2.8	3.48
Wieseria					2.19	2.26			3.52	4.49
Acanthonchus					2.11	2.07				
Megadesmolaimus							2.84	3.35		
Metalinhomoeus							2.54	2.94	2.88	3.24
Theristus									3.88	4.86
Total Taxa : Accumulative %	12	50.68	11	50.63	18	51.61	13	50.53	13	51.16

Table 4.9: Taxa responsible for difference between hydrocarbon treatments in T5 based on SIMPER analysis of square-root transformed data.

T5	Control (82.51)		Clean Lube (80.67)		Used Lube (79.22)		Biodegradable (79.46)		SAB (82.37)	
Taxa	Av.Abund	Contrib%	Av.Abund	Contrib%	Av.Abund	Contrib%	Av.Abund	Contrib%	Av.Abund	Contrib%
Halalaimus	5.26	5.87								
Paracanthonchus	4.03	4.49					3.77	4.52	2.4	2.62
Dichromadora	3.53	4.08								
Paralinhomoeus	3.5	3.89	4.6	6.68			4.26	5.41		
Paramesonchium	3.13	3.18	4.13	5.69	2.64	2.98	4.04	5.26		
Megadesmolaimus	2.67	3.09					2.85	3.39		
Leptolaimus	2.67	3.03			4.2	4.56	3.86	4.29	4.25	5.31
Chromadora	2.69	3.01								
Paramonhystera	2.68	2.82			2.61	2.94	2.43	2.99	2.88	3.24
Linhomoeus	2.67	2.8	4.25	6.3	2.89	3.33			3.12	3.56
Draconema	2.44	2.65	3.93	5.55			3.17	3.96	2.23	2.87
Daptonema	2.48	2.54					2.68	3.08		
Monhystera	2.33	2.52					2.25	2.59	2.77	3.39
Microlaimus	2.29	2.38								
Chromadorina	2.08	2.3	3.46	4.99	2.38	2.87			2.91	2.95
Neochromadora	2.02	2.23								
Pirrickia			5.04	7.32			3.38	4.28		
Desmolaimus			4.13	5.98			2.66	3.29	4.72	5.9
Ixonema			3.56	4.96			2.77	3.27	3.17	3.68
Metalinhomoeus			2.9	3.91						
Trefusia					3.08	4.02				
Theristus					3.13	3.74				
Chromadorita					2.87	3.3			2.21	2.59
Desmodora					2.59	3.2				
Wieseria					2.68	2.86				
Aponema					2.6	2.84				
Promonhystera					2.21	2.83			2.37	2.77
Odonthopora					2.18	2.75	2	2.59		
Chromadorella					2.56	2.74				
Vasostoma					2.46	2.7			3.62	4.61
Acanthonchus					2.33	2.69				
Southerneilla							2.25	2.59	3.47	4.13
Spirobolbolaimus									3.6	4.49
Total Taxa : Accumulative %	16	50.89	9	51.38	16	50.34	14	51.51	14	52.13

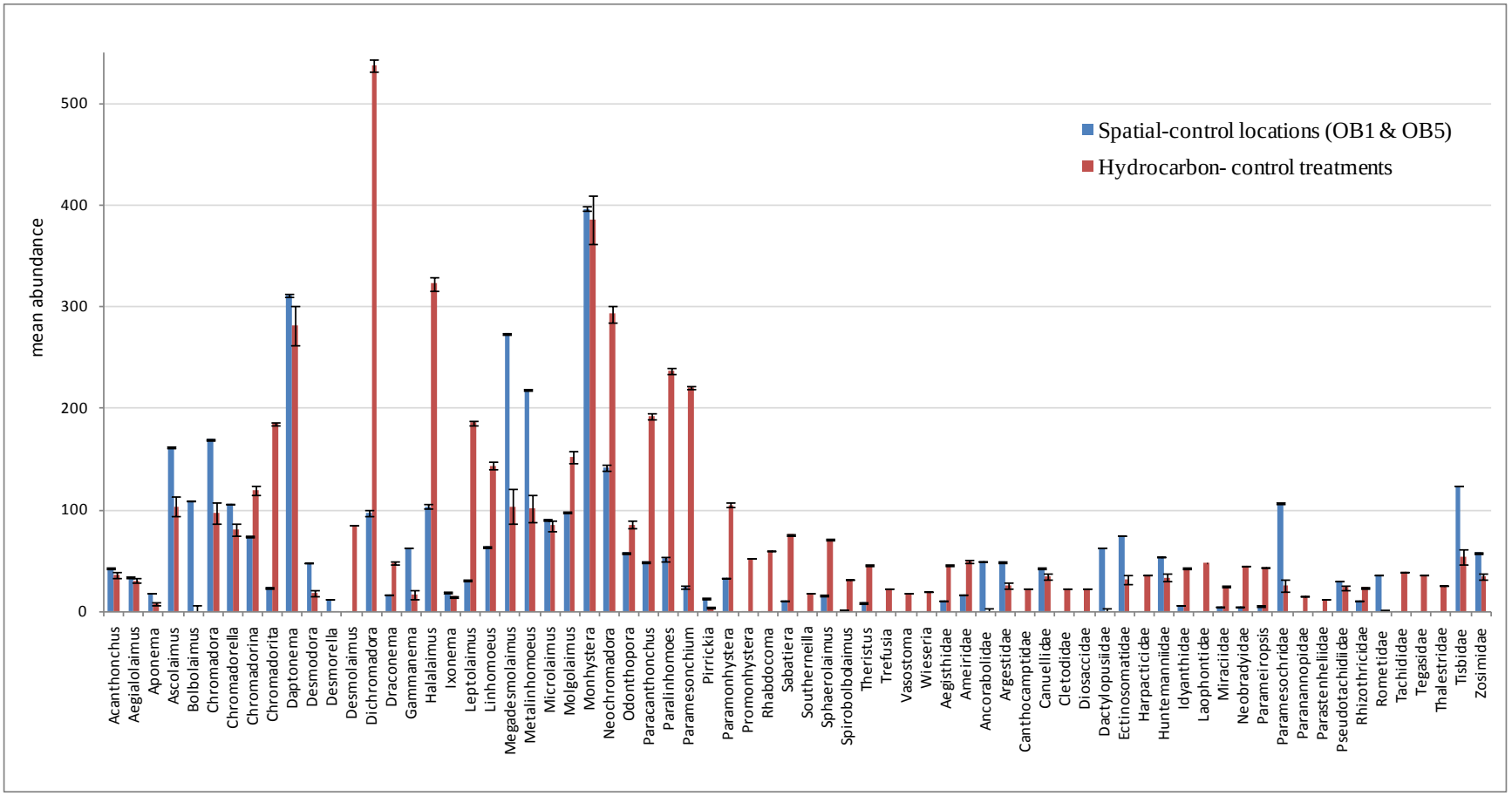

Figure 4.7: Histogram of comparison abundances and taxa between two different studies (control locations in spatial study vs control treatments in hydrocarbon).

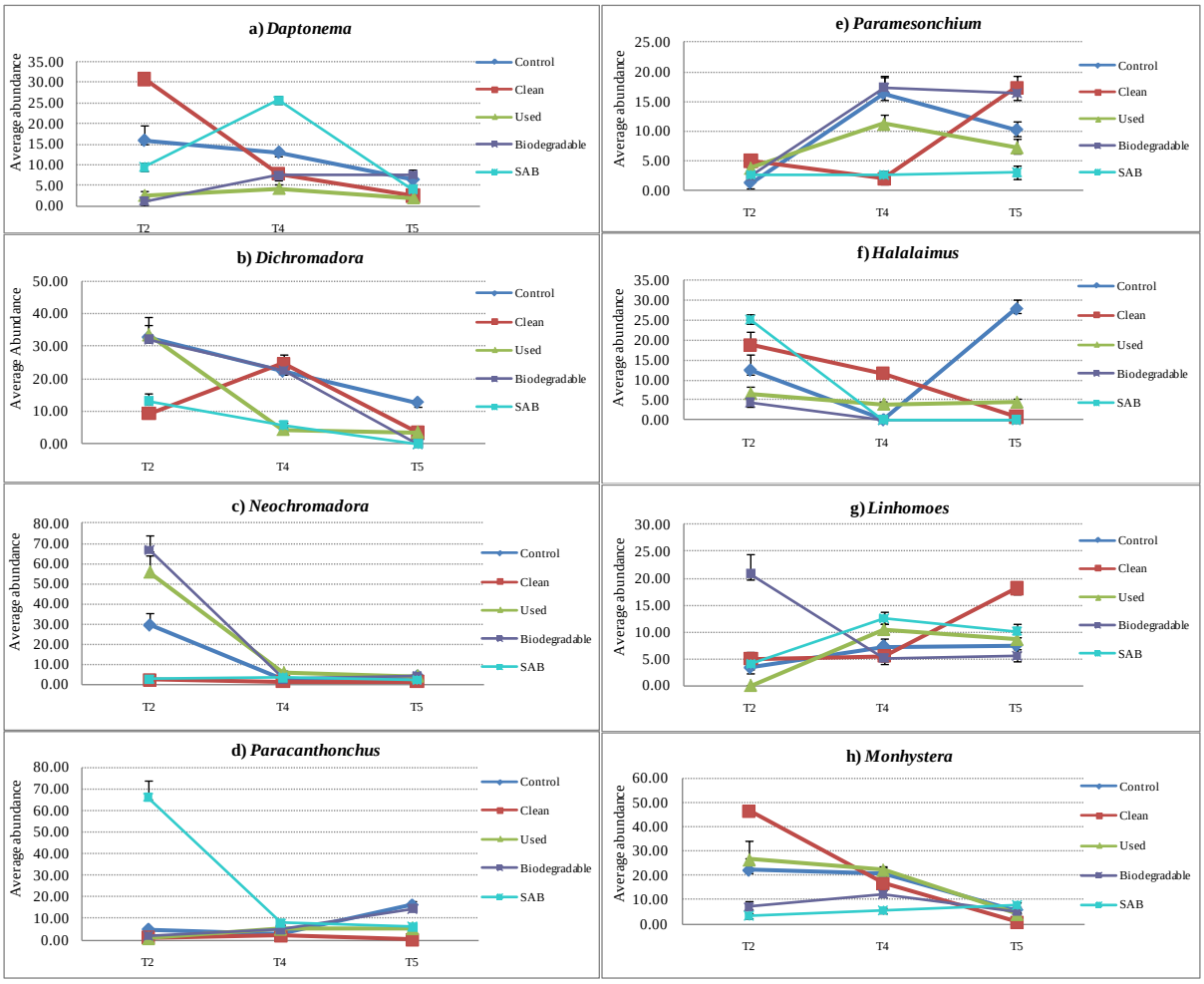

Figure 4.8: Mean abundances of important taxa (summarized from SIMPER results) at each treatment and time.

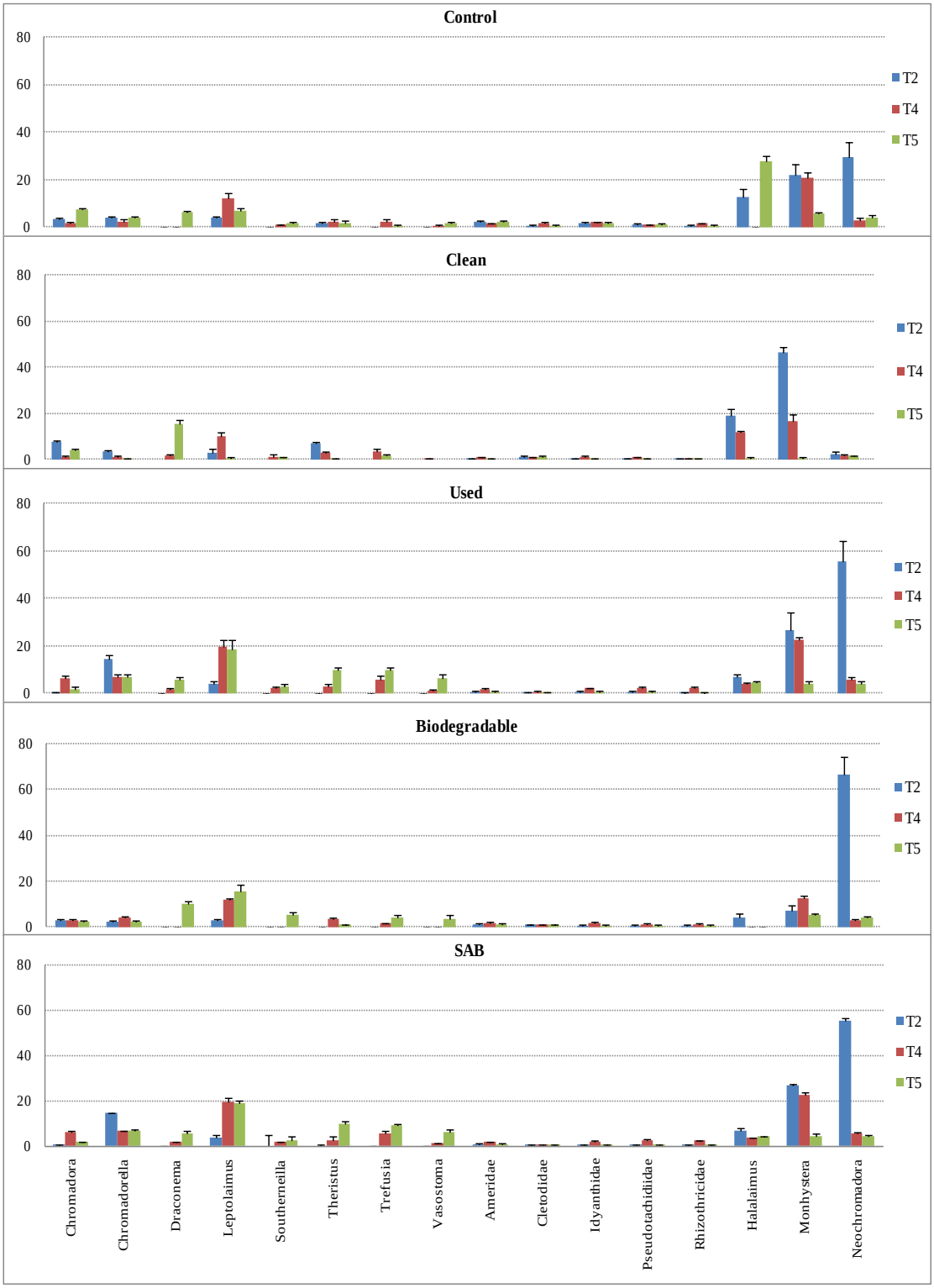

Figure 4.9: Results from BVSTEP analyses showing 16 taxa of the best subset that explained the most variation in meiofaunal composition among treatments (Spearman correlation coefficient $\rho = 0.952$, $P < 0.001$).

4.4 Discussion

Several studies have revealed the sensitivity of meiofauna to various kinds of human activities (Veiga et al., 2009, Beier and Traunspurger, 2001, Bejarano et al., 2006, Moreno et al., 2008) and concluded that meiofauna are good biological indicators for environment impact assessments (Sutherland et al., 2007, Kennedy and Jacoby, 1999, Gyedu-Ababio et al., 1999). The general finding of the above studies is that anthropogenic disturbance and hydrocarbon pollution can alter meiofaunal abundance, species composition, biomass, and diversity. For many individual taxa these alterations are however ambiguous, where the mean values could have either a positive or negative relationship with the impacted and control locations. Furthermore, the impacts are also time dependent and the effects of short-term exposure differs from long term-exposure.

This study is part of a large interdisciplinary project examining the long-term degradation rates of synthetic lubricants and fuel in Antarctic marine sediments and the effects of such lubricants on recruiting infauna (Thompson et al. (2006, 2007), benthic diatom (Cunningham et al., 2003) and microbial communities (Powell et al., 2005b, Powell et al., 2007). This study presents the long term changes in meiofaunal assemblages recruiting to the hydrocarbon contaminated and control sediments from 56 to 260 weeks after deployment. Differences in benthic assemblages between contaminated and control sediments were significant after 56 weeks, with more obvious differences after 260 weeks (Figure 4.7). This indicates that after a hydrocarbon spill, whether fuel such as SAB diesel or oil, impacts to benthic

communities occur quickly and may persist and become more obvious and severe after a longer period. There was no evidence of recovery even after five years suggesting that recovery to pre-spill communities may take a decade or longer.

4.4.1 Loss of oils from sediment

Naturally occurring hydrocarbons in Antarctic marine sediments have been previously reported in a range from 0.1 to 9.3 mg TPH kg^{-1} (Cripps and Priddle, 1991). However, values up to 289 mg TPH kg^{-1} were recorded here in the Control at T3. Initially values in control sediment were at background levels. All sediment for experimentation was collected from unpolluted O'Brien Bay. The increase in TPH in control sediments may be due to resuspension of sediment from nearby contaminated trays. Alternatively it may be due to microbial activity in the sediments.

In this study, large decreases in TPH values were observed by 260 weeks after deployment. A significant loss of 53 to 87% of all oils was observed in the top 1 cm. Biodegradable lubricant oil recorded a loss of up to 87% after 260 weeks. Loss of hydrocarbons from sediment occurs through several processes, especially dilution, dissolution and biodegradation (Powell et al., 2007). A decrease in the TPH values indicates an overall loss of hydrocarbons but the effect on meiofaunal communities is still strong. This effect was clearly seen in the MDS pattern for T5 (Figure 4.5b) as each treatment was distinctively grouped. This may be because compounds contained

within the hydrocarbons, which may also be highly toxic, still remain and may be more difficult to degrade or change over time.

4.4.2 Effects of hydrocarbon contaminated sediment on meiofauna

The effects of hydrocarbons on the meiofaunal assemblages differed between treatments and exposure time. The Clean, Used, and SAB treatments initially had a greater effect than the Biodegradable oil. A significant reduction in mean abundance of meiofauna was observed in those treatments compared to the Control. In contrast, the Biodegradable oil did not have a significant effect on the total abundance of meiofauna (and thus on the total abundance of nematodes) but did have a significant effect on the community composition during the study period. Heip et al. (1985) and Danovaro et al. (1995) observed that high densities of opportunistic nematode species were present after oil spills, probably as a consequence of the increase in bacterial density in the sediments. These bacteria have been found to be an important food source for nematodes after an oil spill (Heip, 1985). This observation is consistent with the findings of Powell et al (unpublished data) on Antarctic benthic microbial communities at T1 (five weeks). In their study total bacterial cell numbers increased in all hydrocarbon treatments compared to the Control. In the SAB and Biodegradable oil treatments an increase in hydrocarbon degrading bacteria was observed after five weeks. The Biodegradable oil did not have strong effect on the microbial community after 56 weeks of exposure but it became more different to the Control after 104 and 260 weeks (Powel, unpublished data). In this experiment the abundance of nematodes did not increase in any of the hydrocarbon treatments, but decreased in most, except

the biodegradable treatment which was not different to the control in terms of abundance. These findings are in contrast to other work which has demonstrated an increase in nematode abundance in response to hydrocarbon contamination (Heip et al., 1985). However, the meiofaunal communities were not sampled at five weeks and it is possible they may have increased initially and this response would have gone undetected. In contrast, the abundance of copepods was lower in the Biodegradable treatment than in the Control at 56 weeks and 260 weeks. Although similar conclusions have been reached by many studies whereby meiofaunal communities are a good indicator of environmental change, many of these studies have yielded different results. Some studies showed that hydrocarbon contamination may influence whole groups of meiobenthic taxa negatively or positively. Generally results obtained from field survey experiments constantly showed acute toxicity effects (reduction in taxa) on meiofauna community immediately after an oil spill (Veiga et al., 2009, Danovaro et al., 1995). While, in laboratory or mesocosm experiment, variability in responses to contamination may occur where increases or reduction in several meiofauna taxa were observed only at certain stages (by time). This trend was observed in this current experiment, whereby indicating a colonization process. Similarly, Fleeger and Chandler (1983) found that several taxa of meiofauna did respond positively to oil application after 90 days of oil introduction and significantly decreases after 150 days. A negative response which is similar to the Casey experiment, was found in a mesocosm experiment conducted by Beyrem et al (2009), which showed a significant decrease in total nematode abundance (I), species richness (d) and number of species (S) after five days exposure. Similar results were found in a study by Frithsen et al. (1985), most metazoan meiofaunal groups showed decreased abundance in the presence of high dosage of oil (average 190 ppb in water column) in

168 days. This variability in responses of meiofauna to oil contamination may be caused by difference in experimental setup and exposure duration.

Fleeger and Chandler (1983) suggested that increase in meiofauna were due to increased bacterial production in oiled sediments, and subsequent heightened copepod grazing. In this study, the only increase in meiofaunal abundance was for copepods in the SAB treatment. However, the SAB diesel treatments showed the most significant decreases in nematode abundances in comparison to other treatments after five years. Powell (2005a) reported that the microbial community in the SAB treatment had changed the most after five weeks and was significantly different from the Control. They suggested that the increase in bacterial numbers was due to the input of carbon from the degradation of the SAB. However, after 260 weeks, no significant differences were observed. They concluded that the hydrocarbon degrading bacteria continued to dominate the community after five years (Powel, unpublished data).

Results of this study indicate that nematodes are generally perhaps more tolerant to hydrocarbon pollution than copepods but that nematodes still clearly respond to hydrocarbon pollution. Similar observations have been reported in other studies (Veiga et al., 2009). High abundances of nematodes were found in most treatment groups. It is well established that nematodes are highly tolerant of stressed environmental conditions, while copepods are more sensitive to disturbance (Mirto et al., 2002). Consequently, the copepod community may be more suitable for short term monitoring due to their sensitivity to pollution. In this study the copepods were generally more sensitive to hydrocarbon contaminated sediments, having a lower

abundance in all treatment groups at 56 and 260 weeks. Similar results were found in Fleeger and Chandler (1983) and Frithsen et al. (1985). These findings are in contrast to those of Thompson et al. (2007) who reported that copepods (> 300 µm) dominated the infaunal assemblages in the contaminated and Control sediments at five and 56 weeks and their recruitment was largely unaffected by the presence of lubricants, but they did not examine the SAB treatment, nor did they identify the copepods beyond this taxonomic level. They reported that the short-term response (five weeks), T1 (ANOVA treatment $F_{3,12} = 3.96$, $p = 0.036$) the abundance in the control was greater than in the oil treatments. After 56 weeks (T2), there were no significant effects. They concluded that the copepods were opportunistic because of their potential for rapid reproduction, which allowed them to dominate the initial stages of faunal succession after a disturbance. An increase in copepod abundance in most of the hydrocarbon treatments at 104 weeks suggests that copepod assemblages may have been responding to some other external factor, as by 260 weeks they all had lower abundances than the control again.

In the Control treatment, some taxa showed a trend of decreasing abundances from 56 to 260 weeks. For example, *Dichromadora and Neochromadora* were dominant at 56 weeks. These taxa were then replaced as the dominant taxa in the control by *Halalaimus* after 260 weeks. *Halalaimus* could be considered as an intolerant to oil contamination since it was eliminated or significantly reduced after 260 weeks of exposure to Clean, Used, Biodegradable and SAB hydrocarbons. In the Biodegradable and Used treatments, *Neochromadora* was initially dominant, confirming its status as opportunistic taxa, but by 104 and 260 weeks it had drastically declined in all treatments where it was initially abundant. Other taxa generally increased in

abundance from 56 to 104 and 260 weeks, including *Paramesonchium*, *Draconema* and *Leptolaimus*. In the Biodegradable treatment, *Daptonema* increased marginally from 56 to 260 weeks but decreases occurred in the other treatments suggesting it may be tolerance to only Biodegradable oil. Beyrem et al. (2009) also reported that *Daptonema* was intolerant to oil contamination, since it was eliminated after five weeks when exposed to hydrocarbons. In other studies, after a long exposure to oil, the sediment hydrocarbon levels decreased, enabling the benthic communities to recover to their normal density and diversity values (Wormald, 1976, Giere, 1979, Bodin, 1988). This was not observed here after the five years of this experiment and strong and obvious effects were still apparent. Similarly, the Clean, Used and SAB showed changes in abundance of many taxa from 56 to 260 weeks. These changes suggest that exposure to long term hydrocarbon pollution may cause changes in community composition, with only tolerant taxa being able to survive. *Monhystera* has been characterized as an opportunistic coloniser genus due to its ability to live in polluted areas (Gyedu-Ababio et al., 1999), but here its abundance generally declined over time in all hydrocarbon treatments where it was initially abundant, showing opportunistic traits. Huge decrease was observed in Clean and Used lubricant treatment. Used lubricants contain metals and additional polycyclic aromatic hydrocarbons that are introduced via engine operation and oil combustion (Thompson et al., 2007). Thompson (2006) suggested that Used lubricant could be biologically degraded as fast as their unused (Clean lubricant) counterpart, where, the alkyl naphthalenes, alpha olefins and alkyl diphenylamines behaved similarly in both Used and Clean treatments. According to Thompson et al. (2007), lubricants contain many toxic compounds such as diphenylamines, alkyl naphthalenes and other minor phenol additives that are not readily biodegradable. Such compounds are very toxic to many

aquatic organisms such as bioluminescent bacteria (*Photobacterium phosphoreum*), amphipods (*Gammarus fasciatus*) and daphnia (*Daphnia magna*) (Drzyzga, 2003). These compounds may remain in sediments after the readily biodegradable fractions have gone and contribute to long term impacts on meiofaunal communities.

Similarities in abundances of meiofauna were observed between the Control and the Biodegradable treatments, which also had the most similar meiofaunal community composition at 56 and 104 weeks. But by 260 weeks, it showed as much difference from the Control as the other hydrocarbon in terms of whole community patterns. These results suggest that prolonged exposure to Biodegradable lubricant will not alter the meiofaunal abundances but will alter community composition. This suggests that Biodegradable lubricant is initially less harmful but may break down into more harmful compounds, which create long term impacts. It may be suitable as an alternative to other oils to minimize the impact of hydrocarbon contamination in Antarctica, as meiofaunal abundances were not affected.

Comparison with Casey macrofaunal assemblage can only be done at T2 in a study by Thompson et al (2007). For example, they found significant reductions in the abundance of many infaunal species in hydrocarbon treatments including cumaceans, amphipods, tanaids and polychaetes after T2 (56 weeks). However, they noted that Evenness (J′) did not differ significantly across all treatments after 56 weeks; Control sediments had a significantly greater Evenness than the Biodegradable treatment, but not the Clean or Used treatments. In contrast, significant differences were observed between all treatments in the meiofauna experiment at T2. Thompson et al (2007) also

concluded that the Biodegradable oil possess the same effect on recruitment of macrofauna as the other conventional lubricants. This is in contrast with meiofauna whereby Biodegradable showed similar responses to the Control treatment. In a relevant study at Casey it was noted that, sediments which were artificially contaminated by hydrocarbons had greater effects on recruitment than those with sediments which were treated with a complex of metals (Stark et al., 2003b). However, in general, the disturbed locations (sites near to Casey Station) had a lower diversity of soft sediment macrofaunal assemblages, but often recorded higher abundances of individuals in hydrocarbon treated sediment by comparison to control (Stark et al., 2003a).While, in undisturbed location such as O'Brien Bay less effect of the hydrocarbon contamination were observed (Stark et al., 2003b).

This experiment was designed to investigate both short (56 weeks) and long-term (260 weeks) effects of diesel fuel and synthetic lubricants on meiofaunal communities. It was observed that each treatment has different effects on meiofaunal communities. Results from this study confirm that changes in nematode assemblages are ideal for long term pollution monitoring as they displayed clear responses to hydrocarbon pollution, both in terms of taxon abundance and community composition. In conclusion, the effects of lubricant contamination on Antarctic meiofauna may be measurable for at least five years after a spill.

5.0: GENERAL DISCUSSION

The Antarctic ecosystem is considered one of the world's most pristine environments, however, the integrity of the environment is threatened by contamination resulting from human activities. The presence of research stations on the Antarctic continent has contributed to the contamination of coastal waters and ecosystems. To date there have been few studies of composition, distribution, and diversity of Antarctic free-living nematode and harpacticoid copepod communities at the species level. This study provides the first general picture of the spatial distribution and structure of the meiofaunal communities and their variability in subtidal coastal waters near Casey Station, East Antarctica. In this thesis, the impact of human activities at a permanently manned research station, Casey, was studied by comparing meiofaunal assemblages at both control and disturbed locations and relating these patterns to sediment contamination and other environmental variables.

A survey of meiofaunal communities was undertaken to determine the spatial variation, abundance, and biodiversity of meiofauna at several scales (Chapter 2). Relationships between spatial patterns of meiofaunal communities and the environment were examined using environmental data such as grain size, organic carbon, and sediment metal concentrations (Chapter 3). In addition, the responses of free-living nematode and harpacticoid copepod communities to hydrocarbon pollution were investigated in an experiment (Chapter 4).

5.1 Summary of findings

The spatial variation of meiofaunal communities at Casey Station was observed between locations (1000's of meters), between sites within locations (100's meters) and between plots within sites (10's of meters). The greatest differences were at the largest scale, between locations, although significant differences in meiofaunal assemblages were found at each spatial scale measured. There were significant differences between the meiofauna at all locations but there was also a very distinctive pattern between control and disturbed locations. Higher abundances of meiofauna were recorded in control locations (O'Brien Bay and McGrady Cove) than disturbed locations (Brown Bay and Wilkes).

In this study, statistical analyses revealed that sediment metal contamination showed significant correlations with patterns of meiofauna distribution and composition. It is proposed that there is a strong relationship between meiofaunal community structure and the extent and type of metal contamination of sediments. In addition to the clear differences between all locations and the potentially strong influence of metals on this pattern there were also clear patterns in the distribution of specific taxa which related to these differences. Some taxa were able to be classified as either opportunistic or sensitive in their relationship to the presence of contamination. For example, the taxa *Halalaimus* and Zosimidae were found to be pollutant sensitive, since they were only present at control locations. This was also found in the hydrocarbon experiment, where *Halalaimus* was found to be sensitive to all hydrocarbon treatments. Two taxa, *Promonhystera* (nematode) and Paramesochridae (harpaticoid copepod), were only

found in the disturbed locations of Brown Bay Inner, Brown Bay Middle and Wilkes and appear to be tolerant of disturbance and could be considered to be opportunistic species. While, the genera *Paramonhsytera* and *Theristus,* which were found to be tolerant to metal pollution, were found be important taxa in Brown Bay.

5.2 The composition of meiofaunal assemblages at Casey, Antarctica

A total of 58 different meiofaunal higher taxa were identified from Casey. The meiofauna assemblage was generally dominated by nematodes (38 genera) and harpacticoid copepods (20 families). The ratio of nematodes to copepods was approximately 4:1. This ratio of nematodes to copepods has previously been observed in other ecosystems, including intertidal mudflats, mangroves, and coastal intertidal areas (Vanaverbeke et al., 1997b, Moreno et al., 2008, Chinnadurai and Fernando, 2007, Ndaro and Ólafsson, 1999, Delgado et al., 2009). This ratio (Ne/Co) has been previously used as a tool in biomonitoring programs based on the argument that nematodes are more pollution tolerant than copepods (Raffaelli and Mason (1981) However, several studies argued that the index, as proposed, was not universally applicable to all habitats (Coull et al., 1981, Lambshead, 1984, Lee et al., 2001b). Peterson et al. (1996) argued that the method could be useful if the differential response of major (even phylum level) meiofaunal taxa to various pollutant stressors, such as sediment toxicants and organic loading, were better understood but Lee at al. (2001b) concluded that the ratio could not be used as a standalone method for

biomonitoring purpose especially on high energy open ocean beaches, due to the low densities of meiofauna and the absence of harpacticoid copepods from the beaches. Furthermore, the abundance of copepod and nematodes and their response to contamination may vary in different habitats. Interestingly, a ratio of 4:1 (Nematodes: Copepods) was consistently observed in both the controlled and disturbed locations at Casey, (Chapter 2).

The dominant taxa were the nematode genera *Monhystera* and *Daptonema*, which were found at all six locations. The most abundant meiofauna taxa, summed over all six locations, were *Monhystera* (11.5%), *Daptonema* (8%), *Neochromadora* (6.3%), Tisbidae (4%), *Odonthopora* (3.6%), *Halalaimus* (3.5%), and *Chromadorina* (3.4%), all of which are nematodes except for Tisbidae. This composition is similar to other Antarctica meiofaunal communities. Most of the nematode taxa found in this study have also been recorded in the southeastern Weddell Sea shelf and slope (200 to 2000 m). These taxa include *Monhystera*, *Daptonema*, *Sabatiera* and *Dichromadora* (Vanhove et al., 1999). In the Weddell Abyssal Plain, nematodes dominated the communities (90%) followed by copepods (5%), with a total of 17 taxa. Similarly, Lee (2001a) notes that *Monhystera* was the dominant taxon out of 38 taxa found in a study of Kapp Norvegia, Weddell Sea. *Monhystera* is known to have a worldwide distribution (Alongi and Tietjen, 1990, Gerlach and Schrage, 1971, Vanreusel et al., 2000, Vanaverbeke et al., 1997a, Vanhove et al., 1999) and has been found abundantly in both organically enriched and polluted sediments (Lorenzen et al., 1987, Gyedu-Ababio et al., 1999) and is known as a colonizer genus and is considered an indicator of pollution (Veiga et al., 2009). The genus *Monhystera* also has the highest abundance at the disturbed location of Brown Bay, Casey (Chapter 2).

In addition to *Monhystera*, *Daptonema* was also found to be abundant at Casey. This genus, *Daptonema,* has also been reported as one of the most abundant genera in the shallower areas (230m) of the deep, Arctic Laptev Sea (Vanaverbeke et al., 1997a). Tisbidae, a marine benthic copepod, had a high abundance at the Wilkes location. This family is commonly found between depths of 20-200 m (Seifried, 2004) and is widely distributed in communities adjacent to the Ross Ice Shelf (Bradford and Wells, 1983), Weddell Sea (Dahms, 1989, Günther et al., 1999, Schunemann et al., 2008) and the South Orkney Islands (Gee and Fleeger, 2008), Antarctica.

Sabatiera and *Daptonema* are typically found in organically rich, muddy sediments (Heip et al., 1990, Schratzberger et al., 2006) and are thought to be well adapted to disturbed conditions (Vanreusel, 1990). In particular, the genus *Sabatieria* survives at low oxygen concentrations and high sulphide concentrations and often persists under conditions that are unsuitable for most other nematode species (Hendelberg and Jensen, 1993, Steyaert et al., 1999, Tietjen, 1980). At Casey, *Sabatiera* was found at the disturbed location, Brown Bay, but was least abundant at the control location, O'Brien Bay. In contrast, *Daptonema* was dominant at McGrady Cove, which had a high proportion of coarse sand and is the control location in Newcomb Bay. *Daptonema* has also been found in a heavily polluted harbour in Ligurian Sea, which is characterized by silt and muddy sediments (Moreno (2008). It is known that the sediment type affects meiofauna composition. For example, Doulgeraki et al. (2006) noted that meiofauna are often more abundant and diverse at sites with coarse sediments where water circulates more freely, resulting in a better oxygenation of the substrate, a condition which would also allow a deeper penetration of meiofauna. This suggests that both the presence of pollution and sediment type contributes to the

meiofaunal community composition and distribution. The composition of meiofauna at Casey is similar to most studies in the Antarctic and other regions and has a similar relationship to sediment grain size and pollutants.

5.3 The effects of heavy metal and hydrocarbon contamination on meiofaunal communities

The field survey indicates that Antarctic meiofauna are sensitive to elevated metal concentrations contributed from both anthropogenic and non-anthropogenic sources (Chapter 3). Findings from this study not only suggest that heavy metal pollution from activities at Casey Station reduces meiofaunal abundance but that it may also alter the composition. In a previous study, it was demonstrated that there were higher concentrations of some heavy metals in sediment collected from bays adjacent to Casey Station (Brown Bay and Wilkes) in comparison to control locations which were situated further away (Cunningham et al., 2005, Stark et al., 2003b). The presence of these pollutants was correlated with patterns of differences in macrofaunal assemblages (Stark et al (2003b) in a similar manner to meiofaunal communities.

Anthropogenic contamination of marine sediments was obvious at some disturbed locations such as Brown Bay. However, Wilkes, a potentially disturbed location, had metal concentrations and meiofaunal assemblages similar to the control locations. The Wilkes waste dump site is much older than the Casey site and has not been disturbed

recently and also does not have a melt stream running through it. The concentration of most metals was similar to the controls except for a few, Cd and Zn and to a lesser degree, Pb and Sn, which were slightly higher. Meiofaunal assemblages at Wilkes were similar to those of the control sites (O'Brien Bay) in some ways but in other ways resembled a disturbed location, suggesting that perhaps it was impacted at some point in its past since human occupation. Wilkes may have recovered from pollution impacts if they existed in the past, or there may be other unmeasured contaminants present..

Higher concentrations of heavy metals are typically associated with the fine-grained fraction of the sediment rather that the coarse-sized fractions (Tam and Wong, 2000), although this difference becomes less significant when the region becomes more contaminated. In this current study, Brown Bay, which is the most disturbed location, had the highest fraction of fine sediment and the highest metal concentrations. While O'Brien Bay, located well away from the Station, had coarser sediments and much lower metal concentrations. McGrady Cove, the control location in Newcomb Bay, also recorded high concentrations of some metals, such as Ag, As, Cd, Ba and Th, and it also had fairly fine sediments. This suggests that fine sediment could enhance the effects human impacts in the presence of heavy metals. Wilkes, however, had coarser sediments more similar to the control sites, which may have influenced the metal concentrations measured there. Coarse sediments have been found to increase the toxicity of sediment metals, so that lower concentrations can be as toxic as at sites with fine grained sediments and higher concentrations (Pesch 1979).

Most studies of meiofauna and metal toxicity have been conducted in microcosm experiments (Mahmoudi et al., 2007, Ellis et al., 2001, Austen and McEvoy, 1997, Millward and Grant, 2000, Millward et al., 2001, Fichet et al., 1999, Lee et al., 2001b, Ser, 1991, Beyrem et al., 2007). These laboratory experiments have tended to show that metals as toxicants have either lethal or sublethal effects, which tend to increase with increased metal concentrations (Somerfield et al., 1994). A decrease in diversity is therefore to be expected as a community responds to metal pollution. In our field study, such a conclusion could not be made since the effects were from a combination of a number of heavy metals and other interacting environmental factors, however, changes in the community structure were still observed. Furthermore, nMDS analysis showed that there was a distinctive pattern of meiofauna between disturbed and control locations. This is in an agreement with Tietjen (1980), who found that there was a clear relationship between metal concentrations and decreased nematode diversity.

Human activities in the Antarctic are heavily dependent on petroleum and synthetic oil products for transport, power, and heating. Wide ranges of lubricating oils are used in vehicles and heavy machinery. Given the harsh operating conditions and the quantity of oil that is used in the Antarctic, there is a significant risk of spills entering the marine environment. Two different type of hydrocarbons commonly used at Casey Station (SAB diesel fuel, and a Clean lubricant oil) as well as the same clean lubricant after use in machinery (Used Lubricant) and a potential alternative to conventional petroleum based lubricants, a Biodegradable lubricant oil, were examined to determine their effects on sediment meiofaunal communities. Each of the four hydrocarbons was found to have a strong effect on the meiofaunal communities at

Casey even after five years, when significant biodegradation of the contaminants had occurred in the top layer of sediment. Duration of contaminant (hydrocarbon) exposure was shown to be important, as at each sampling time different effects on the meiofaunal assemblages were observed. For example, the biodegradable oil appeared to have little effect for the first few years but by five years it had a strong effect on communities (Chapter 4). The changes demonstrate that exposure to long term hydrocarbon pollution will cause changes in community composition, where tolerant taxa are able to survive and dominate assemblages. Results from this study suggest that changes in nematode assemblages are ideal for long term pollution monitoring as they displayed some very clear responses to hydrocarbon pollution, particularly in terms of their abundance.

In polluted areas, a few tolerant or opportunistic taxa will become relatively more numerous and dominate the community, while less tolerant species will become increasingly rare or disappear (Moreno et al., 2009, Gyedu-Ababio et al., 1999, Gyedu-Ababio and Baird, 2006). In this hydrocarbon experiment, changes in species composition were observed, for example, in all oil treatments, *Halalaimus* was present at the early stage of the experiment (T2), however, this taxa become rare (in Used Oil) and disappeared (in other oils) after prolonged exposure (T5). Taxa such as *Neochromodora* were seen as an early colonizing, opportunistic species and was only abundant at early stages, and was reduced after 260 weeks. Observation of species elimination was also observed by Mahmoudi et al (2005) in a microcosm experiment, whereby gradual changes in community structure were revealed, when a quantity of diesel (ranging from 0.5 to 20 mg diesel kg^{-1}) was administrated. Species such as

Chaetonema were eliminated after 90 days and *Hypontolaimus colesi* and *Daptonema trabeculosum* dominated the contaminated sediment.

In a monitoring program after an accidental spill of diesel fuel at Faraday Research Station, Galindez Island, Antarctica in March 1992, it was observed that there were high *n*-alkane and PAH concentrations in the seawater near the station immediately after the spill but these decreased to local background concentrations within a week (Cripps and Shears, 1997). The diesel spill had an immediate toxic effect in the intertidal zone but the fuel dispersed quickly, due to evaporation, dissolution, and dispersal. However, concentrations in the animals living close to the station never decreased to the levels found at the uncontaminated control site (Cripps and Shears, 1997). Similarly, results from this thesis showed that while there was a decrease in TPH after five years, the impacts on the meiofaunal communities were still apparent. Vincx and Heip (1991) concluded that while there is usually an obvious decrease in nematode abundance after an oil spill, and after a year, a huge increase in a few opportunistic species could occur. A similar trend was observed in this study where the abundance of several taxa decreased or were replaced after being treated with hydrocarbon for 48 weeks (Chapter 4).

5.4 Comparison of meiofauna as a biological indicator with other marine benthic communities

There are several characteristics that enable meiofaunal communities to be used as biological indicators. For instance, this community responds rapidly to perturbation, possesses a close relationship with the sediment matrix and has a short life cycle (Kennedy and Jacoby, 1999, Moreno et al., 2008). Meiofauna may seem to have more advantages as indicator species than other benthic invertebrates as they are confined to the substrate throughout their life cycle. Although having advantages, there are also several drawbacks of using meiofauna for environmental monitoring (Table 5.1). Benthic diatoms apparently have similar advantages to the meiofaunal community as they also have short regeneration times and respond quickly to environmental changes (Cunningham et al., 2005). Despite this, there is little information on the impact of petroleum hydrocarbons and metal contamination on benthic marine diatoms. Cunningham et al. (2003), Cunningham and McMinn (2004) and Cunningham et al. (2005) showed that benthic diatom communities were affected by both hydrocarbon and metal contamination at Casey Station. Although responses to pollution may be different between benthic diatom and meiofaunal communities, both communities are sensitive to disturbance and anthropogenic pollution.

While the meiofauna have some advantages as indicator species compared to macrofauna (Table 5.1), studies have shown that some macrofauna are also reliable in indicating the presence of pollution. For instance, Thompson et al (2007) demonstrated that lubricant contamination reduces recruitment to Antarctic soft-

sediment communities for at least one year. For example, they found significant reductions in the abundance of many infaunal species in hydrocarbon treatments including cumaceans, amphipods, tanaids and polychaetes after T2 (56 weeks). Relationships between hydrocarbon and metal contamination with macrofauna have also been observed in many other studies (Stark et al., 2003c, Stark, 1998, Saunders et al., 2007, Pearson and Rosenberg, 1978, Gregory, 2007, Bustos-Baez and Frid, 2003, Ahn et al., 1996). Austen and Widdicombe (2006) showed that the meiofaunal and macrofauna communities had similar, strong responses to the interactive effects of physical disturbance and productivity. However, Kennedy and Jacoby (1999) suggest that meiofauna are more sensitive to heavy metals than macrofauna even at lower concentrations. By comparing results from this current study with others on macrofauna at Casey Station it could be concluded that both meiofauna and macrofauna showed similar responses to contamination. For example, Stark et al. (2003b), reported that soft sediment assemblages (> 1 mm) were closely correlated with concentrations of heavy metals in sediments at Casey Station. Similar correlation was observed in this study (Chapter 3).

Table 5.1: Advantages and disadvantages of using meiofauna for environmental monitoring (Obtained from Kennedy and Jacoby (1999)).

No.	Advantages	Disadvantages
1.	Small size and high abundance mean that even small samples will contain sufficient total individuals for statistical tests	Small size makes counting and identifying meiofauna a difficult task. Microscope required.
2.	High species richness means that the information content of meiofaunal samples is high	Diversity and lack of taxonomic expertise complicates meiofaunal classification
3.	Ubiquitous distribution means that meiofauna can continue to be used as pollution indicators where macrofauna do not occur	Spatial and temporal variability of meiofaunal populations complicates separation of natural and anthropogenic changes
4.	Rapid generation time means that sub-lethal impacts may be detected more rapidly in meiofauna communities than among macrofauna	Resilience of meiofauna to impacts makes them less suitable indicators unless changes in diversity are considered
5.	Direct benthic development and sessile habit mean that meiofauna provide an integrated picture of impacts at a site	Extraction of meiofauna from samples is time-consuming and may lead to uncorrectable errors (i.e. artefacts)
6.	Meiofauna show a particularly dramatic response to chemical pollution, but are relatively insensitive to physical disturbance, enabling chemical impacts to be distinguished from physical ones	'Psychological problem' (sensu Giere, 1993) of basing a monitoring programme on the study of microscopic organisms whose importance in maintaining overall ecosystem integrity is not widely appreciated

5.5 Conclusions

There have been a number of previous studies of benthic marine pollution in Antarctica to monitor and understand the ecological consequences of human disturbance on the benthic community (Lenihan and Oliver, 1995, Lenihan, 1992, Cripps and Shears, 1997, Duquesne and Riddle, 2002, Green and Nichols, 2004, Saul et al., 2005). The human disturbances at Casey Station are caused mainly by routine research station operations (such as sewage, small fuel and oil spills and emissions), past waste disposal practices and shipping. Previous studies of shallow subtidal areas near Casey Station have monitored the impacts on benthic ecosystems by studying changes in benthic microbial, microalgae and macrofauna communities (Thompson et al., 2006, Powell et al., 2005b, Stark et al., 2003c, Cunningham et al., 2005, Cunningham et al., 2003, Stark et al., 2006, Stark et al., 2003b, Stark et al., 2003a, Powell et al., 2005a, Thompson et al., 2007). By examining impacts on the meiofaunal community, this thesis contributes to the understanding of the effects of ongoing human impacts at Casey Station. It is apparent that there are effects on benthic communities from station activities at all levels examined, from the microscopic to the macroscopic.

The station activities at Casey have contributed to the contamination of the local benthic ecosystem. Previous work on the benthic fauna in the marine environment surrounding Casey station has shown differences in the infaunal communities that were correlated with the presence of pollutants. The higher concentrations of contamination are known to occur in sediments collected from bays adjacent to Casey

as compared to control locations located further afield (Stark et al., 2003b, Snape et al., 2001, Cunningham et al., 2003). The contamination had caused change to the benthic composition in many ways. Powell et al. (2003), in the microbial community, heavy metals also appear to have some influence on the communities, particularly iron, cadmium, manganese, zinc and arsenic. As for hydrocarbon contamination, Powell (2005b) reported only minor changes occurred after 5 weeks in the microbial communities in the sediment treated with Biodegradable, SAB, Clean and Used lubricants. Their short-term experiment showed that SAB diesel appears to have caused the biggest change in microbial component among other lubricant used. This short-term changes were also observed in the diatom composition, whereby difference in abundance between control and hydrocarbon contaminated treatment were visible after 11 weeks exposure (Cunningham et al., 2003). Diatom community composition also differed at disturbed locations in comparison to the control locations (Cunningham et al., 2005). They noted that, in Brown Bay, metal concentration particularly those of copper, iron, tin and lead are responsible for the large proportion of variation in diatom community composition. Similarly, meiofaunal communities in disturbed locations near Casey Station were different but had lower abundances than at control locations. Similar findings have been found at other research stations around the Antarctic continent, for example at McMurdo Station, the benthic sediment is heavily contaminated with anthropogenic chemicals and the benthic community is dominated by polychaete worms, which are opportunistic species commonly found in polluted harbor habitats (Lenihan and Oliver, 1995, Lenihan, 1992). In general, the disturbed locations (sites near to Casey Station) had a lower diversity of soft sediment macrofaunal assemblages, but often recorded higher abundances of individuals (Stark et al., 2003b). It may be concluded that the presence

of contamination from the station activities causes the benthic community structure to alter and reduces the biodiversity.

Different taxa responded differently to pollution and at least one group from each community could be considered as an indicator taxa. Thus, presence or increase of particular taxa may indicate contamination. For example taxa such as *Orchomenella franklini* (gammarid) was very abundant in Brown Bay (impacted site) and was very low or nearly absent in control sites (Stark et al., 2003c). This organism has low abundance in O'Brien Bay which has less hydrocarbon and metal contamination by comparison to Brown Bay where levels of hydrocarbons and metals are high. Similarly in studies on diatom, Cunningham et al. (2005) found out that *Navicula* were the indicator for the impacted sites. For example, *N. directa* and *N. aff. glacei* showed a positive correlation with contamination in Brown Bay. Although diatoms have been proven as an indicator for impacted sites, caution should be made in making conclusion as species compositions were also affected by grain size and effects of hydrocarbons also varies between types of hydrocarbon involved (Cunningham et al., 2005). While, in this study on meiofauna, both genera from nematodes *Odonthophora* and *Paralinhomoes* were considered as pollutant indicators. Thus, monitoring the abundance of these taxa could be useful in future monitoring programmes. By selecting particular groups or taxa to monitor, time consuming laboratory work (identification of all organisms in community) could be avoided. However, concentration of contaminants and other environmental variables should be considered in all monitoring programmes.

Based on the monitoring studies at Casey station, it can be concluded that benthic communities living in sediments are well-suited for environmental impact detection and monitoring in Antarctica. They have a fixed spatial relationship with the source of impact by comparison to those organisms which are mobile and/or live in the water column. Whereby mobile organisms are able to avoid stressful condition by moving out of the impacted area.

5.6 Future research

In this current study, the monitoring and evaluation of human impacts were based on the meiofaunal community, providing useful information about the environmental conditions at Casey Station. Furthermore by studying the meiofauna community at Casey, a much broader context for environmental assessments has been obtained.

This study found some strong correlations between patterns of meiofaunal assemblages and metal concentrations in sediments. However these are only correlations and further work in the form of laboratory and/or field experiments are needed to test the hypothesis that metals alter meiofaunal community structure and composition. In particular meiofauna appear to be sensitive to Ag, As, Ba, Cd, Pb, Sn, U, and Zn. Experiments using single metals or combinations of several metals would be useful in testing whether it is metals and not some other environmental variable influencing the observed patterns.

This study was not able to measure hydrocarbons in sediments and for future work it would be useful to test whether there are also strong correlations between meiofaunal assemblage patterns and the presence of hydrocarbons. Although hydrocarbons were proven to affect communities in the field experiment, hydrocarbons are likely to co-occur with metal pollution and it is uncertain which would have the greatest influence on meiofaunal assemblages.

Meiofauna are a component of the benthic ecosystem that responds clearly to anthropogenic disturbance as well as natural environmental fluctuations. Monitoring meiofauna in environmental assessments would be a useful tool to include in such studies in Antarctica.

To further study the effects of heavy metal and hydrocarbon pollutants on meiofauna, it is recommended to use an individual taxa or species as well as experiments with whole communities. Further questions that warrant investigation include:

1. At what concentration does metal contamination affect individual taxa and the meiofaunal community?
2. How do different species respond to different levels of contamination?
3. Do meiofaunal communities recover from contamination and how long is the recovery period?
4. Does the combination of multiple contaminants have synergistic effects that are greater than single contaminants?

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